Regulation of Proton Coupled Electron Transfer from Amino Acids in Artificial Model Systems: A Mechanistic Study

BY

MARTIN SJÖDIN
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Abstract

Amino acid radicals are key redox intermediates in several natural enzymes including Cytochrome c peroxidase, DNA photolyase, ribonucleotide reductase, cytochrome c oxidase and photosystem II. Electron transfer from amino acids is often coupled to deprotonation and this thesis concerns the coupling of electron transfer from tyrosine and tryptophan to trishypyridineruthenium(III) with deprotonation in model complexes. Specifically the mechanisms for these proton coupled electron transfer reactions have been studied and the controlling parameters have been identified, the possible mechanisms being stepwise electron transfer followed by deprotonation and deprotonation followed by electron transfer or concerted electron transfer/deprotonation.

Proton coupled electron transfer reactions have been studied using nano-second flash photolysis in water solution and the effect of pH, temperature, reaction driving force, deuterium and nature of the amino acid has been determined. I have shown that the rate constant for the concerted reaction depends intrinsically on the mixing entropy of the released proton and that the pH-dependence can be used as an experimental tool for mechanistic discrimination. Moreover I have shown that the concerted reaction inherently has a high reorganisation energy due to the coupling of the electron motion with deprotonation. Hydrogen bonding to the transferring proton however significantly reduces this reorganisation energy. The concerted reaction also has a relatively high driving force counteracting the high reorganisation energy in the competition between the concerted reaction and the stepwise electron transfer first reaction. The relative importance of the high reorganisation energy and the high driving force for the concerted reaction determines the mechanistic outcome of the reaction, the stepwise reaction being favoured by high over-all driving forces and the concerted reaction by high pH.

By comparing my results from model complexes with tyrosineZ oxidation in photosystem II, I give strong evidence for a concerted electron transfer/deprotonation mechanism.

Keywords: Proton Coupled Electron Transfer, Tyrosine, Tryptophan, Electron Transfer, Hydrogen bond, Photosystem II, Proton transfer, Radical protein

Martin Sjödin, Department of Physical Chemistry, Box 579, Uppsala University, SE-75123 Uppsala, Sweden

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List of Papers

This thesis is based on the following papers and will be referred to in the text by their Roman numerals.

I. Proton Coupled Electron Transfer from Tyrosine in a Ruthenium-tris-bipyridine Complex: Comparison with Tyrosine\textsubscript{Z} Oxidation in Photosystem II

II. The Mechanism for Proton Coupled Electron Transfer from Tyrosine in a Model Complex and Comparisons with Y\textsubscript{Z} oxidation in Photosystem II

III. Switching the Redox Mechanism: Benchmarks for Proton Coupled Electron Transfer from Amino Acids
    Sjödin, M., Styring, S., Wolpher, H., Sun, L., Hammarström, L. Paper in Manuscript

IV. Hydrogen-bond effects on Proton-Coupled Electron Transfer from Phenols

V. Proton Coupled Electron Transfer from Tyrosine: Tuning the Competition Between a Concerted and a Step-wise Mechanism
   Sjödin, M., Ghanem, R., Polivka, T., Pan, J., Styring, S., Sun, L., Sundström, V., Hammarström, L. Paper in Manuscript
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IV
En Mekanistisk Studie rörande Reglering av Protonkopplad Elektronöverföring från Aminosyror i Artificiella Modellsystem

I fotosyntesen omvandlas solenergi till kemisk energi i form av energirika föreningar, kolhydrater (ekv. 1). Som substrat i reaktionen används endast vatten, koldioxid samt solljus. Förutom biomassa bildas syrgas, som är nödvändig för vår egen existens, som en biprodukt i reaktionen.

\[ 6CO_2 + 6H_2O + nhv \rightarrow C_6H_{12}O_6 + 6O_2 \]  

(1)

Människan är helt beroende av fotosyntesen då större delen av vår föda produceras av växter direkt eller indirekt. Även vår energiproduktion baseras till stor del på förbränning av de produkter som bildats i fotosyntesen. I dagens energikrävande sammhälle är den outtömliga tillgången på solljus och vatten mycket tilltalande för framtida energiproduktion.

I Konsortiet för Artificiell Fotosyntes utnyttjas den kunskap som finns om den naturliga fotosyntesen för att skapa artificiella system som på ett effektivt sätt kan omvandla solens energi till kemisk energi i form av ett bränsle. Liksom i det naturliga systemet ska vatten användas som substrat, men istället för att producera biomassa vill man producera ett enkelt bränsle t.ex. vätsgas (ekv. 2) som sedan kan förbrännas (ekv 3).

\[ 2H_2O + nhv \rightarrow 2H_2 + O_2 \]  

(2)

\[ 2H_2 + O_2 \rightarrow 2H_2O + energi \]  

(3)

när det oxiderats fyra gånger genom de fotoinducerade elektronöverföringsreaktionerna oxideras vatten till syrgas. Denna vattenoxidation reducerar manganklustret tillbaks till sitt ursprungstillstånd och en ny cykel kan påbörjas.

För att på ett effektivt sätt kunna hårma reaktionerna som sker i fotosyntesen i artificiella system är det naturligtvis av stor betydelse att identifiera de nödvändiga komponenterna för energiomvandling i fotosyntesen och förstå deras funktion. Det naturliga systemet är dock mycket komplicerat och därför svårundersökt. I denna avhandling har istället enkla modellsystem använts för att förstå en av elementarreaktionerna i fotosyntesen, den primära återreduktionen av pigmentet, det vill säga elektronöverföringen från tyrosin till det oxidera pigmentet.

Av central betydelse för vilken funktion tyrosinet kan tillskrivas i fotosyntesen är dess protoneringstillstånd. Då den reducerade formen av tyrosin, ursprungstillståndet, är en tämligen svag syra (pKa = 10) och den oxiderade formen är en mycket stark syra (pKa = -2), är oxidation av (elektronöverföring från) tyrosin kopplad till deprotonering av aminosyran vid fysiologiska pH.

Figur 1: Elektronöverföring från tyrosin är kopplad till deprotonering av tyrosinets OH-grupp. Dessa två elementarreaktioner kan mekanistiskt ske på tre distinkt skilda vis: Oxidation och deprotonering kan ske stegvis med elektronöverföring eller deprotonering som primärt steg (ETPT' respektive PTET') eller samtidigt (CEP). Dessa två kopplade elementarreaktioner, elektronöverföring och deprotonering, kan mekanistiskt ske på tre distinkt skilda vis (figur 1). Elektronöverföringen och deprotoneringen kan ske konsekutivt med elektronöverföringen eller deprotoneringen som primärt reaktionssteg (ETPT' respektive PTET') eller ske simultant i ett reaktionssteg (CEP). Genom modellsystemen har jag kunnat visa att den omgivande vattenlösningens pH påverkar samtliga mekanismer olika. Detta har sedan använts som huvudsakligt verktyg för att betstämma vilken mekanism som dominerar i modellsystemen. Vidare har de parametrar som styr konkurrensen mellan mekanismerna identifierats och kvantifierats. Ur detta har framgått att reorganisationsenergin och drivkraften för reaktionen är de
styrande parametrarna i konkurrensen mellan den samtidiga mekanismen, CEP, och den konsekutiva mekanismen med ett primärt elektronöverföringssteg, ETPT. (I ETPT är den initiala elektronöverföringen (ET) hastighets-begränsande).

På grund av kopplingen av elektronöverföringen till deprotonering i CEP har denna mekanism en stor reorganisationsenergi. Mot detta står att kopplingen till deprotonering även ger en högre drivkraft, som ökar med omgivningens pH. Detta gör det möjligt för den samtidiga mekanismen att konkurrera med den sekventiella mekanismen trots dess inneboende höga reorganisationsenergi. Den mekanism som dominerar beror på vilken av dessa två parametrar som har störst betydelse, den högre reorganisationsenergin för CEP eller den lägre drivkraften för ET.

För att bättre modellera situationen i biologiska system där tyrosinets proton är vätebunden till en eller flera baser har även effekten av vätebindningar på protonkopplad oxidation av vätebundna fenoler undersöks. Av betydelse för konkurrensen mellan de olika mekanismerna i denna studie är att ETPT mekanismen inte påverkas märkbar av vätebindningen medan CEP blir betydligt effektivare. Den högre effektiviteten för CEP i ett vätebundet system beror troligtvis på att den höga reorganisationsenergin för denna mekanism reduceras av vätebindningen.

Genom jämförelse med den tyrosinoxidation som sker i fotosyntesen i naturliga system, där manganklustret avlägsnats, med modellsystemen har jag kunnat visa att elektronöverföring och deprotonering sker simultant i en CEP-mekanism. Vidare betonar studien vikten av vätebindningar mellan tyrosinets proton och närliggande baser för tyrosinets funktion som en effektiv elektrondonator till det oxiderede pigmentet.
Abbreviations

A  Electron acceptor
B  Base
CEP  Concerted electron transfer/deprotonation reaction
D  Electron donor
e  electron charge
E\textsuperscript{0}  Standard potential
E\textsuperscript{0}′  Formal potential
E\textsubscript{a}  Activation energy
EnT  Energy transfer
ET\textsuperscript{ox}  Oxidative quenching
ETPT\textsuperscript{′}  Sequential electron transfer followed by deprotonation
ET\textsuperscript{red}  Reductive quenching
h  Planck’s constant
HOMO  Highest occupied molecular orbital
Ka  Dissociation constant
k\textsubscript{B}  Boltzmann constant
k\textsubscript{d}  deprotonation rate constant
k\textsubscript{p}  Protonation rate constant
L  Link between electron donor and electron acceptor
LC  Ligand centered (transition)
LUMO  Lowest unoccupied molecular orbital
MC  Metal centered (transition)
MLCT  Metal-to-ligand charge transfer (transition)
MV\textsuperscript{2+}  Reduced Methyl viologen (Viologen radical)
MV\textsuperscript{2-}  Methyl Viologen
OEC  Oxygen evolving complex
PCET  Proton coupled electron transfer
PSI  Photosystem I
PSII  Photosystem II
PTET\textsuperscript{′}  Sequential proton transfer followed by electron transfer
Q  Quencher
[Ru(bpy)\textsubscript{3}]\textsuperscript{2+}  trisbipyridineruthenium(II)
Ru(II)-Ala  Ru(II)(bpy)\textsubscript{2}(4-Me-4′-(CONH-L-alanine ethyl ester)-2,2′-bpy)
Ru(II)-TrpH  Ru(II)(bpy)\textsubscript{2}(4-Me-4′-(CONH-L-tryptophan ethyl ester)-2,2′-bpy)

VIII
Ru(II)-TyrOH  \( \text{Ru}(\text{II})(\text{bpy})_2(4\text{-Me-4'}\text{-(CONH-L-tyrosine ethyl ester)-2,2'}\text{-bpy}) \)

RuEster(II)-TyrOH  \( \text{Ru}(\text{II})(4,4'\text{-di-COOEt-2,2'}\text{-bpy})_2(4\text{-Me-4'}\text{-(CONH-L-tyrosine ethyl ester)-2,2'}\text{-bpy}) \)

SHE  \( \text{Standard Hydrogen Electrode} \)

\( T \)  Absolute temperature

\( \text{Trp}^- \)  Deprotonated tryptophan

\( \text{Trp}^* \)  Deprotonated oxidized tryptophan (radical)

\( \text{TrpH}^- \)  Tryptophan

\( \text{TrpH}^* \)  Protonated oxidized tryptophan (radical)

\( \text{TS} \)  Transition state

\( \text{TyrO}^- \)  Deprotonated tyrosine, tyrosinate ion

\( \text{TyrO}^* \)  Deprotonated oxidized tyrosine (radical)

\( \text{TyrOH}^- \)  Tyrosine

\( \text{TyrOH}^* \)  Protonated oxidized tyrosine (radical)

\( \chi \)  Vibrational wave function

\( \Delta G^0 \)  Standard free energy change of reaction

\( \Delta G^0_{\text{a}} \)  Free energy of activation

\( \Delta G^{0'} \)  Formal free energy change of reaction

\( \varepsilon \)  Extinction coefficient

\( \varepsilon^0 \)  Dielectric constant

\( \varepsilon_{\text{op}} \)  Optical dielectric constant

\( \varepsilon_s \)  Static dielectric constant

\( H_{\text{dp}} \)  Electronic coupling energy

\( \lambda \)  Reorganization energy

\( \lambda_{\text{in}} \)  Inner reorganization energy

\( \lambda_{\text{out}} \)  Solvent reorganization energy

\( \nu \)  Frequency of light

\( \nu_n \)  Frequency of passage through the transition state
1 Introduction

Photosynthesis, the conversion of carbon dioxide to energy rich carbohydrates, utilises solar energy as energy source to drive the complex endergonic reactions in eq. 1.1. Light-energy is thereby converted to chemical energy, in the form or glucose, which can be used by living organisms. Organisms can metabolise glucose but not light. In oxygenic photosynthesis dioxygen, crucial for our own existence, is produced by oxidation of water as a by-product in the reaction. Water that is one of the most abundant molecules on earth thus acts as an infinite electron source in the photosynthetic reaction.

\[ 6\text{CO}_2 + 6\text{H}_2\text{O} \rightarrow C_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \quad (\Delta G^\circ = 2870 \text{ kJmol}^{-1}) \]

The photosynthetic machinery is situated in the thylakoid membrane inside the chloroplast in cells of green plants and algae.\(^1,2\) Two large protein-cofactors each containing a photosystem, one with an absorption maximum at 700 nm and the other at 680 nm, spans the membrane and hosts the reactions of the light induced reactions. In the two photosystems, which are called photosystem I (PSI) and photosystem II (PSII), the primary step is transfer of a photo-excited electron from a pigment, P700 and P680 for PSI and PSII respectively, to an electron transfer chain (figure 1.1).

---

Figure 1.1: Electron-flow and approximate reduction potentials in Photosystem I and in Photosystem II. The solid arrows indicate electron transfer reactions while the segmented arrows correspond to light absorption.
The electron transfer initiated in PSII ultimately leaves the electron at the PSI site. Here the electron again gains energy by excitation of P700 and enters a second electron transfer chain that reduces a ferrodoxin. The reduced ferrodoxin in turn donates an electron to NADP$^+$ to form NADPH that is used to bind and reduce carbon dioxide to glucose in the Calvin cycle.

PSI is supplied with electrons from PSII. PSII on the other hand relies on its own catalytic ability to oxidize water photo-chemically to dioxygen as source of electrons. Light-driven oxidation of water thus provides the whole photosynthetic process with electrons. With the endless supply of water this reaction has, since the first oxygenic photosynthetic reaction appeared for about three billion years ago, fuelled nearly all living organisms on earth with energy originating from sun and water.

In the energy-demanding society of today the everlasting supply of energy from sun and water is appealing. In the Swedish consortium for artificial photosynthesis the knowledge in natural photosynthesis, that is the only system known today that is able to oxidise water with light as only energy source, is employed to construct synthetic molecules with the aim to perform light driven water oxidation. The accomplishment of such reaction would provide society with a pure source of electrons, the only by-product being dioxygen and protons, that could be utilised as current or as an electron source for fuel production.

However much is still unknown about the natural photosynthetic machinery and this thesis is devoted to the understanding of one of the important elementary reactions in photosystem II, the oxidation of tyrosine. With the complexity of a protein matrix the study of a single reaction event in the sequence of reactions occurring in photosynthesis is a formidable task. Instead, in this work, simple model systems are used to understand the mechanism by which tyrosine may be oxidised.

A very important aspect of electron transfer from tyrosine is the protonation states involved in the reaction and the coupling of the electron motion with proton motions. In this thesis the mechanisms of proton coupled electron transfer reactions from tyrosine and tryptophan in model compounds have been studied in detail and revealed the parameters that controls the outcome of the competition between different mechanisms. These findings could be used as guidelines for mechanistic discrimination of tyrosine oxidation in PSII. The general results are also applicable to proton coupled amino acid oxidation in other radical proteins. In many enzymatic redox reactions the importance of tyrosine- and tryptophan radical intermediates has become increasingly apparent (See for example ref. 5).

To better model the actual situation in a protein, where hydrogen bonds hold together the protein matrix and most likely also affect the tyrosine proton$^+$, oxidation of hydrogen bonded phenols have been studied that elucidates the effect of hydrogen bonds in proton coupled electron transfer reactions.
Photosystem II (PSII) is the heart of natural photosynthesis. It pumps electrons through the photosynthetic reaction centres and catalyses light-driven oxidation of water (for a review see for example ref. 2, 7, 8 and 9). PSII is a 300 kDa protein complex, made up of more than 25 protein subunits and at least 13 redox cofactors, that spans the thylakoid membrane (figure 2.1). The most important subunits are the D1 and D2 intertwined proteins that contain all redox cofactors involved in the electron transfer chain as well as the catalytic water oxidation site, the oxygen-evolving centre (OEC). (In early days of photosynthesis, it is believed, the D1 and D2 were identical but by the course of evolution the roles of the two subunits changed). Chlorophyll-containing proteins surround this heterodimer. These proteins absorb light and transfer the excitation energy to the P680 chlorophylls in the D1 core. Due to their energy-collective function they are called antenna proteins.

Figure 2.1: Schematic structure of the Photosystem II reaction center. Electron transfer reactions are indicated as arrows.

Excitation of P680 by light-energy starts an electron transfer-chain reaction. The electron situated on one of the four chlorophylls building up P680 transfers to a primary pheophytin (Pheo) electron acceptor in 2-21 ps. The charges separated in this charge-separated state, P680⁺-Pheo⁻, recombine in 30-40 ns. However, competing with recombination a second electron transfer from Pheo⁻ to the plastoquinone Q₅, occurs within a few hundred ps stabilising the charge separation. Also this state, P680⁺-Pheo⁻Q₅⁺, is unstable.
with respect to charge recombination, $\tau \approx 100 \mu s$. A redox active tyrosine, tyrosine$_2$ (Y$_2$), on the donor side of PSII prevents electron transfer from Q$_A^-$ to P$_{680}^-$ by re-reducing the oxidised primary donor. On the acceptor side the electron is transported further to Q$_b$. When reduced twice, in a second light induced electron transfer from P$_{680}$ via Q$_A$, Q$_b$ takes up two protons and leaves its D1 binding site as Q$_b$H$_2$ and is replaced by a second plastoquinone from the quinone pool ($Q_{pool}$) (figure 1.1). The released plastoquinone (Q$_b$H$_2$) then interacts with cytochrome bf, that catalyses electron transfer from the plastoquinone to the plastocyanine (PC) protein. This mobile PC protein passes the electrons on to the PSI reaction centre. As a consequence of the electron transfer reactions, oxidation equivalents are built up at the donor side of PSII. These oxidation equivalents end up at the oxygen-evolving complex (OEC) where it is utilised to oxidise water.

2.1 The oxygen evolving complex

Oxidation of water to dioxygen requires removal of four electrons (or build-up of four oxidation equivalents) but the electron transfer reactions induced by light absorption of one photon only provide the OEC with one oxidation equivalent. One of the main objectives of the oxygen-evolving complex (OEC) therefore is to store oxidation equivalents through four photo-induced one-electron transfer events.

Figure 2.2: In the S-cycle the oxygen evolving complex (OEC) is taken from its dark stable state, S$_1$, through five different redox states (S-States) by light induced one-electron transfer from OEC to the oxidised sensitizer P$_{680}^-$. In the transition from S$_4$ (the most oxidised state) to S$_0$ (the most reduced state) oxygen is evolved.

This is done in the so-called S-cycle introduced by Kok in the early 70:s (figure 2.2). The S-cycle was introduced to explain the complex pattern of oxygen release when dark-adapted PSII particles were exposed to saturating light-flashes; It was found that oxygen was evolved first after the third flash
and thereafter after every fourth flash.\textsuperscript{14} In the S-cycle the OEC is taken from its dark stable state, S\textsubscript{1}, through five oxidation-states by light-induced electron transfer through tyrosine\textsubscript{Z} to the P680-site back to its original redox state. At the transition from S\textsubscript{4}, the most oxidised state, to S\textsubscript{0}, the most reduced state, oxygen is released.

The core of the oxygen-evolving centre (OEC) is a manganese cluster consisting of four manganese ions.\textsuperscript{15} Several structures for the manganese cluster has been proposed, based mainly on EPR\textsuperscript{16,17} and X-ray absorption studies\textsuperscript{18} and also on low-resolution X-ray structures\textsuperscript{19,20}, but none without ambiguity. Amongst the most popular are a dimer-of-dimer\textsuperscript{16} structure however recently a 3 + 1 tetramer has gained popularity\textsuperscript{15}. The 3 + 1 structure is also supported by the highest resolution X-ray structure available.\textsuperscript{20} Also the valence of the manganese ions in the S-cycle are under debate the dominant suggestions being Mn(III)Mn(III)Mn(IV)Mn(IV) and Mn(III)Mn(III)Mn(III)Mn(III) in the dark stable state.\textsuperscript{16,21} In all steps in the S-cycle, except for the S\textsubscript{2} to S\textsubscript{3} transition where ambiguity remains\textsuperscript{22}, the manganese cluster is oxidised and thus oxidation equivalents are stored in the Mn-cluster. In the S\textsubscript{3}-state ligand oxidation, other than water, is considered as an option to Mn-oxidation and therefore no water oxidation occurs prior to the formation of the short-lived (1 ms) S\textsubscript{4}-state according to current models.

2.2 The function of Tyrosine\textsubscript{Z}

The function of tyrosine\textsubscript{Z} as a redox intermediate between P680\textsuperscript{+} and the OEC is indisputable.\textsuperscript{23,24,25} However the mechanisms by which tyrosine\textsubscript{Z} is oxidised by P680\textsuperscript{+} and reduced by the manganese cluster are not fully understood nor are the protonation states of tyrosine involved in the electron transfer reactions.

At all pH where PS II is active the reduced form of tyrosine\textsubscript{Z} is protonated in PSII.\textsuperscript{26,27} However, the tyrosine radical formed by electron transfer to P680\textsuperscript{+} is very acidic and unstable with respect to deprotonation. Oxidation of tyrosine\textsubscript{Z} by P680\textsuperscript{+}, that occurs predominantly in 50 ns in the S0 and S1 states and in 250 ns in S2 and S3,\textsuperscript{2} is therefore coupled to deprotonation. Without the aid of deprotonation, electron transfer from the protonated form of tyrosine to P680\textsuperscript{+} is energetically unfavourable, $\Delta G^0 \approx 0.3$ eV, (ET-step, Figure 2.3).\textsuperscript{28,29} Thus, a sequential electron transfer followed by deprotonation (ETPT') has a highly endergonic primary electron transfer step. It is therefore unlikely that this electron transfer reaction could give rise to the sub-μs electron transfer rates that efficiently prevents charge recombination between QA\textsuperscript{−} and the oxidised primary donor.

Instead the dominant view is that electron transfer from tyrosine originates from tyrosinate oxidation (ET'-step, Figure 2.3) and thus that...
Deprotonation occurs prior to the electron transfer event (PT-step figure 2.3).\textsuperscript{30,31} This step-wise mechanism (PTET') gives an exergonic electron transfer, $\Delta G^0 \approx -0.4$ eV.\textsuperscript{29} However, since the protonated form of tyrosine Z is stable the primary, deprotonation, reaction is still energetically unfavourable. For the free form of tyrosine the rate of deprotonation to water is very slow at neutral pH, $\tau \sim 100$ ms (See section 5.1 and 5.4), and the over-all rate for this step-wise mechanism can not exceed the rate of any of the individual steps, PT or ET'. By the addition of a base however the deprotonation rate can be increased substantially.\textsuperscript{32} In PSII tyrosine Z deprotonation is facilitated by a hydrogen bond to a near-by base (B), presumably histidine 190.\textsuperscript{33,34} In histidine 190-free mutants the rate of P680' reduction was significantly slowed-down in support of this idea.\textsuperscript{33,34,32}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.3.png}
\caption{Possible mechanisms for proton coupled electron transfer from tyrosine Z to P680' in PS II. In the upper scheme an endergonic electron transfer reaction (ET) is followed by deprotonation (PT') of the tyrosine radical in a step-wise ETPT' mechanism. The lower scheme shows a step-wise mechanism where deprotonation occurs prior to electron transfer. This mechanism gives an exergonic electron transfer from tyrosinate to P680'. Deprotonation of tyrosine is facilitated by a hydrogen bond to a base (B). Apart from the two sequential mechanism, ETPT' and PTET', a concerted electron transfer/deprotonation reaction (CEP) is possible (middle scheme). In this mechanism electron transfer and deprotonation occurs simultaneous in one single reaction event.}
\end{figure}

Although the step-wise deprotonation-first (PTET) mechanism seems to be a strong candidate for tyrosine Z oxidation it is not without ambiguity. The maximum rate constant for the model is given by the fraction of tyrosinates (x) times the electron transfer rate constant ($k_{ET}$) in the non-proton-limited case (eq. 2.1).

$$k_{PTET} = xk_{ET}$$  \hspace{1cm} (2.1)

A non-proton transfer limitation has been dominating the discussion in the literature due to the absence of deuterium isotope effects in the intact
system\textsuperscript{32,35} and effectively means that the proton transfer reactions, PT and -PT, are fast compared to electron transfer, ET'. Due to the fast proton transfer reactions a pre-equilibrium between tyrosine and tyrosinates is established. In the hydrogen bond situation depicted in figure 2.3 the fraction of tyrosinates is given by the difference in pKa between tyrosine and the base (eq 2.2).

\[ x = 10^{-(pKa(Tyrosine) - pKa(B))} \quad (2.2) \]

In order to account for the 50 ns kinetics, with a pKa for histidine of about 7 and a tyrosine pKa of 10, the electron transfer rate constant would have to be \(2 \times 10^{10} \text{ s}^{-1}\). However the rate constants for tyrosinate oxidation by P680\textsuperscript- in intact systems at pH where the tyrosine is initially deprotonated are in the range of \(10^6\) to \(10^7 \text{ s}^{-1}\).\textsuperscript{32} This reaction corresponds to the electron transfer reaction (ET'). Clearly this discrepancy has to be explained for the proton transfer first-model to hold.

In a low-dielectric medium, such as a protein, charged species are poorly stabilised and therefore high in energy. The sequential mechanisms (ETPT' and PTET') both give charged tyrosine intermediates that react further in secondary stabilising reactions to give a neutral tyrosine radical. A third mechanism for tyrosine\textsubscript{2} oxidation would be a concerted electron transfer-deprotonation reaction (CEP, figure 2.3). This mechanism is appealing because charged tyrosine intermediate states in the low dielectric protein matrix are avoided. Since the primary reaction and the stabilising reaction occurs simultaneously this mechanism is energy conservative in that the free energy of the reaction is integrated in a single step. In spite of the intuitive advantages with the concerted model this mechanism has been somewhat over-looked in the PSII community and therefore not exposed to experimental scrutiny.\textsuperscript{36} A better understanding of this mechanism is required in order to test the concerted model for tyrosine\textsubscript{2} oxidation.

For the re-reduction of tyrosine\textsubscript{2} on the other hand a concerted electron transfer/protonation reaction, or hydrogen atom transfer, has gained much attention during the last ten years.\textsuperscript{2,37,38} The interest arises from the proposal that tyrosine\textsubscript{2} is an integrated part in the OEC in that it is directly involved in the catalytic oxidation of water.\textsuperscript{39} In this model tyrosine in its oxidised, deprotonated form act not only as an electron acceptor for the Mn-cluster but also as a proton abstractor from manganese bound water. In support of this model, at least for the last step in water oxidation, oxygen release occurs on the same time-scale as tyrosine\textsubscript{2} reduction.\textsuperscript{2} Many mechanistic questions are still unexplained by the hydrogen atom abstraction model and a unifying picture have not yet appeared for the tyrosine\textsubscript{2} redox reactions. (See for example ref. 36).
In this chapter the individual parts building the supra-molecular complexes used as model systems for proton coupled electron transfer reactions are described. The super molecule consists of a sensitizer (reaction initiator) that absorbs visible light and is in most cases covalently linked to an electron donor known to undergo deprotonation upon oxidation under certain conditions. Hence electron transfer from the donor is coupled to deprotonation. A sufficiently long bridge between the donor and the sensitizer separates the electron-transfer units and decreases their interaction thus enabling separate treatment of the individual units.

In order to induce an electron deficiency into the sensitizer part of the supra-molecule an electron acceptor that quenches the sensitizer excited state oxidatively is introduced, corresponding to the electron transfer chain from P680 to the quinones. The quencher is not bound to the supra molecule but undergoes a bimolecular reaction which makes it possible to ensure sufficiently fast quenching rates by the use of large quencher concentrations while keeping recombination reactions slow.

Due to its very favourable chemical and photo-physical properties (see below) trisbipyridineruthenium(II), [Ru(bpy)$_3$]$^{2+}$, and derivatives of [Ru(bpy)$_3$]$^{2+}$ have been used as sensitizers in all experiments. (In comparison with PSII reactions the sensitizer has the same function as the chlorophyll pair P680). Apart from the favourable properties of [Ru(bpy)$_3$]$^{2+}$ the complex is also well known and has been extensively studied so that its properties are well established.$^{40,41}$

To model proton coupled electron transfer reactions, tyrosine (TyrOH) and tryptophan (TrpH) units have been used as electron donors to the photodised ruthenium sensitizer. These amino acids are well known as redox active components in many biological systems and an understanding of their electron transfer reactions is of specific interest in many systems.$^5$ For example in Cytochrome c peroxidase a redox active tryptophan is believed to mediate electron transfer.$^{42}$ In DNA photolyase$^{43}$, ribonucletide reductase$^{44}$, cytochrome c oxidase$^{45}$ and in PS II instead tyrosine redox chemistry is of importance. (Actually, in DNA photolyase and in cytochrome c peroxidase tryptophan and tyrosine are possibly involved in electron transfer reactions between each other).$^{46,47}$ Although these redox active amino acids are known
to be important as electron transfer mediators the mechanism by which they operate is under debate. In this thesis tyrosine and tryptophan in simple model compounds serve as a basis for a more general discussion of proton coupled electron transfer that could be useful for the understanding of the redox processes that occur in complex biological systems.

The end of this chapter briefly explains the experimental technique, time resolved flash photolysis with transient absorption detection, which has been used to measure electron transfer rates from the donor to the oxidised sensitizer. Importantly the technique requires that the reactants undergo absorption changes during the reaction to be detected.

3.1 Trisbipyridineruthenium(II) as Sensitizer

Ru$^{2+}$ is a d$^6$ ion that with strong field polypyridyl ligands forms particularly stable low spin complexes i.e. the t$_{2g}^6$-state is the most stable electron configuration. The stability of the Ru$^{2+}$-polypyridyl complexes can be traced to the low energy π* ligand orbitals that interact with the metal t$_{2g}$ orbitals thus stabilising the t$_{2g}$ while increasing the energy for the π$_L$*-orbital.$^{41}$ Depending on the specific polypyridyl ligand the π$_L$*- ligand orbitals may be higher or lower in energy than the e$_g$ metal d-orbitals. In trisbipyridineruthenium(II) (figure 3.1) that is a octahedral complex the π$_L$*- orbitals are lower in energy than e$_g$ (figure 3.1) and thus corresponds to the lowest unoccupied molecular orbital (LUMO). Whilst the LUMO level is mainly ligand in character the highest occupied molecular orbital (HOMO), t$_{2g}$, is metal centred.

![Figure 3.1: Structure (right) and schematic molecular orbital diagram (left) of trisbipyridineruthenium(II) with absorption transitions indicated as arrows.](image)

Promotion of an electron from HOMO to LUMO therefore corresponds to a charge transfer between the metal and the ligand. Since the electron in the LUMO resides on the ligands other molecules can readily reach it and
interact with the electron. Depending on the nature of the molecule (Q) interacting with the $[\text{Ru(bpy)}_3]^{2+}$ excited state the dominant reaction can be oxidative- or reductive quenching ($\text{ET}^{\text{ox}}$ and $\text{ET}^{\text{red}}$ respectively) of the excited state or energy transfer (EnT) from the excited complex (eq. 3.1 – 3.3). In this thesis only the oxidative quenching path is of interest since this reaction induces the desired electron deficiency at the ruthenium sensitizer site and the quencher has to be selected accordingly.

$$
*\text{[Ru(bpy)]}_3^{2+} + Q^{\circ} \rightarrow \text{[Ru(bpy)]}_3^{3+} + Q^{-} \quad \text{ET}^{\text{ox}}
$$  \hspace{1cm} (3.1)

$$
*\text{[Ru(bpy)]}_3^{2+} + Q^{\circ} \rightarrow \text{[Ru(bpy)]}_3^{3+} + Q^{+} \quad \text{ET}^{\text{red}}
$$  \hspace{1cm} (3.2)

$$
*\text{[Ru(bpy)]}_3^{2+} + Q^{\circ} \rightarrow \text{[Ru(bpy)]}_3^{2+} + *Q \quad \text{EnT}
$$  \hspace{1cm} (3.3)

Figure 3.2: Deactivation paths for the excited state of trisbipyridineruthenium(II): Radiative and non-radiative transitions are represented by solid arrows and dotted arrows respectively. The rate constants represents intersystem crossing from the singlet state to the triplet state ($k_{\text{isc}}$), activated transition from the MLCT triplet state to the MC triplet state ($k_{d-d}$), non-radiative deactivation of the $^3\text{MLCT}$ ($k_{\text{nr}}$) and radiative transition from $^3\text{MLCT}$ to the ground state ($k_{r}$) respectively.

In competition with oxidative bimolecular quenching the excited state also undergoes internal deactivation (figure 3.2). Excitation of the metal-to-ligand charge transfer band (MLCT) gives the singlet $^1\text{MLCT}$-state. This state rapidly, $\tau < 1$ ps, relaxes to the triplet state ($^3\text{MLCT}$) with unit probability through intersystem crossing. $^{41,48,49}$ Relaxation of the $^3\text{MLCT}$ to the ground state occurs through three different reaction paths: non-radiative relaxation ($nr$), radiative relaxation ($r$) and activated transition to the $e_g$-state (d-d) giving the metal centred triplet d-d state ($^3\text{MC}$) followed by rapid non-radiative relaxation to the ground state. In non-polar solvents formation of the $^5\text{MC}$ state can lead to photo-substitution of the ligands. $^{50}$ The over-all rate constant ($k^{0}$) for the relaxation process is given by the sum of rate constant for the different reaction paths (eq 3.4) and in water solution it is about $1.5 \times 10^6$ s$^{-1}$ corresponding to a lifetime of the excited state of 650
Thus, in order for the quenching process to be efficient, the rate of bimolecular quenching has to be significantly faster than 650 ns which is readily accomplished. Radiative relaxation gives rise to a strong phosphorescence, $\Phi_{em} = 0.04$, with a maximum at 600 nm that can be used to monitor the excited state decay. A decrease in emission lifetime and emission intensity by addition of the quencher indicates efficient quenching of the excited state.

$$k^0 = k_r + k_{nr} + k_{d-d}$$

(3.4)

As stated in the introduction of this chapter the signal in transient absorption measurements are due to changes in optical density as a consequence of photo-induced reactions. Figure 3.3 shows the absorption spectrum for [Ru(bpy)$_3$]$^{2+}$. In the visible region the MLCT dominates the spectrum ($\lambda_{max} = 452$ nm, $\epsilon_{452} = 14600$ M$^{-1}$cm$^{-1}$)$^{41}$. A second MLCT transition occurs at 240 nm. The large UV-absorption peak corresponds to a ligand centred (LC) transition, $\pi_L - \pi_L$. In between the LC transition and the 450-nm MLCT transition two weak absorption bands corresponding to metal centred (MC) d-d transitions can be seen. In this work the strong MLCT absorption has been used to generate the excited state. The high extinction coefficient ensures that large concentrations of excited state molecules are formed that improves the signal.

![Absorption spectrum for the [Ru(bpy)$_3$]$^{2+}$ ground state in water solution. The different transitions are marked with vertical lines and the identities of the transitions are indicated above the respective absorption peak.](image)

Figure 3.3: Absorption spectrum for the [Ru(bpy)$_3$]$^{2+}$ ground state in water solution. The different transitions are marked with vertical lines and the identities of the transitions are indicated above the respective absorption peak.

Excitation of the complex dramatically reduces the extinction coefficient of the MLCT transition ($\epsilon_{452} \sim 5000$ M$^{-1}$cm$^{-1}$ for $^5$MLCT)$^{51}$ and thus excitation induces a strong negative transient absorption signal. Also the oxidised complex [Ru(bpy)$_3$]$^{3+}$ absorbs less then the ground state [Ru(bpy)$_3$]$^{2+}$ ($\epsilon_{452} \sim 2000$ M$^{-1}$cm$^{-1}$)$^{40}$ and gives rise to a negative signal.
Following the 450-nm absorption it is therefore possible to monitor the excited state formation and decay as well as formation of flash-quenched produced oxidised complexes and its concomitant redox reactions, i.e. electron transfer reactions regenerating the \([\text{Ru(bpy)}_3]^{2+}\) ground state (see below).

![Figure 3.4: Modified trisbipyridineruthenium(II), Ru(II)(4,4'-di-COOEt-2,2'-bpy)$_2$(4-Me-4'-(CONH-L-tyrosine ethyl ester)-2,2'-bpy) (Ru Ester(II)-TyrOH), with two electron withdrawing ester groups attached to two of the bipyridine ligands. In this complex a tyrosine unit (in gray) is attached to the non-ester-substituted ligand.](image)

The \([\text{Ru(bpy)}_3]^{2+}\)-sensitiser absorbs strongly in the visible region enabling formation of large concentrations of excited states long-lived enough to be oxidatively quenched by external acceptors. Moreover the excited state reactions as well as the ground state redox reactions can be monitored by time resolved flash photolysis with transient absorption detection. Another important feature of the \([\text{Ru(bpy)}_3]^{2+}\)-sensitiser is the possibility of altering the redox potential by modifying the bpy ligands. The reduction potential of \([\text{Ru(bpy)}_3]^{3+/2+}\) in water solution is 1.26 eV vs. NHE$^{51,41}$ which is close to the reduction potential of the PS II sensitizer P680 ($E^0(P680^+/P680) = 1.12$ eV$^{29}$). Introducing two electron withdrawing ester groups on two of the bpy ligands (figure 3.4) increases the reduction potential by 0.25 eV. Thus by minor alterations of the ligands the redox properties of the \([\text{Ru(bpy)}_3]^{3+}\)-sensitizer can be significantly altered enabling fine-tuning of the electron transfer energetics.

3.2 Electron Acceptors

The main objective of the electron acceptors in this thesis is to oxidise the excited sensitizer fast enough to give a well-defined time zero for the following reaction events without further involvement in the reactions. Moreover it is advantageous if the acceptor does not interfere with the measurements. In this respect \([\text{CoCl(NH}_3)_3]^{2+}\) is an obvious choice. This acceptor quenches the \([\text{Ru(bpy)}_3]^{3+}\) excited state oxidatively with a rate
constant of $7.2 \times 10^7$ M$^{-1}$s$^{-1}$ and decomposes to form non-reactive decomposition products, Cl$^-$, NH$_4^+$ and Co$^{2+}$ (eq 3.5). Moreover the reactants and products does not absorb strongly in the visible region why it does not give any transient absorption signals that could interfere with the time resolved transient absorption measurements. Unfortunately the benefits of using sacrificial acceptors is counteracted by same irreversibility that gives the advantages. Since the acceptor is unable to return the electron to the system investigated oxidation equivalents will build up, as an effect of the measurement, that could interfere with a second measurement on the same sample. Moreover, the concentration of initial reactants will decrease for every measurement performed on the system. Averaging of several measurements, necessary to get reliable data, is difficult using irreversible acceptors for these reasons.

$$[*\text{Ru(bpy)}_3]^{2+} + [\text{CoCl(NH}_3 \text{)}_5]^{2+} \rightarrow [\text{Ru(bpy)}_3]^{3+} + \text{Cl}^- + 5\text{NH}_3 + \text{Co}^{2+}$$

(3.5)

A reversible acceptor on the other hand ensures that the system returns to its initial state after each measurement assuming of course that the system investigated undergoes reversible reactions itself (eq 3.6-3.7). For kinetic studies this back-reaction gives an undesired reaction path for the oxidised $[\text{Ru(bpy)}_3]^{2+}$ back to its initial redox state (eq. 3.7) that has to be controlled. A compromise between the desired non-interference and control of the recombination reaction thus has to be made. If the acceptor is invisible in the measurement giving no signals that could interfere with the transient absorption read-out there is no way of controlling its back reaction rate.

$$[*\text{Ru(bpy)}_3]^{2+} + \text{MV}^{2+} \rightarrow [\text{Ru(bpy)}_3]^{3+} + \text{MV}^{2+}$$

(3.6)

$$[\text{Ru(bpy)}_3]^{3+} + \text{MV}^{2+} \rightarrow [\text{Ru(bpy)}_3]^{2+} + \text{MV}^{2+}$$

(3.7)

![Methyl viologen](image)

Figure 3.5: The structure of Methyl viologen, used as an oxidative quencher to the excited trisbipyridineruthenium(II) sensitizer.

For the $[\text{Ru(bpy)}_3]^{2+}$-system methyl viologen (figure 3.5) is a good compromise between these parameters. The doubly charged oxidised form (MV$^{2+}$) is colourless, while the viologen racial (MV$^{2+}$) spectrum (reduced form) covers most part of the visible region with absorption maximum at 396 nm and 606 nm. However, in the spectral region between 420 nm
and 490 nm where \([\text{Ru(bpy)}_3]^{2+}/[\text{Ru(bpy)}_3]^{3+}\) gives strong transient absorption signals the viologen radical absorption is weak. This allows for the recombination reaction (eq. 3.7) between \([\text{Ru(bpy)}_3]^{3+}\) and the methyl viologen radical to be followed separately from the reaction between \([\text{Ru(bpy)}_3]^{3+}\) and the amino acid. This electron acceptor is also fast with a diffusion controlled rate for oxidative quenching (eq. 3.6) of the \([\text{Ru(bpy)}_3]^{2+}\) excited state \((k_q = 5.6 \times 10^9 \text{ M}^{-1}\text{s}^{-1})\). If the spectral region where the methyl viologen radical absorbs is of interest hexaamineruthenium(III), \([\text{Ru(NH}_3)_6]^{3+}\), can be used as a complement to the methyl viologen acceptor. \([\text{Ru(NH}_3)_6]^{3+}\) is also a reversible acceptor but contrary to methyl viologen it gives no transient absorption signals in the visible region thus opening up spectral regions that are masked by methyl viologen radical absorption.

### 3.3 Tyrosine and Tryptophan as Electron Donors

Tyrosine and tryptophan are both well-known redox active amino acids (figure 3.6). The only stable form of the two amino acids at neutral pH is the neutral protonated reduced form. The oxidised radical form is unstable with respect to dimerisation. Upon oxidation they undergo deprotonation and thus electron transfer from the two compounds is coupled to deprotonation. The coupling of oxidation to deprotonation is reflected in the pH-dependence of the formal potential (figure 3.6). Because the mixing entropy of the released proton increases as the pH increases the reduction potential decreases as a consequence of the coupled deprotonation reaction with 59 meV per pH. The formal reduction potential \((E^{0'})\) is 0.93 V and 1.015 V for tyrosine and tryptophan respectively at pH 7 thus oxidation of both compounds are thermodynamically accessible with the \([\text{Ru(bpy)}_3]^{3+}\) sensitizer at this pH.

![Figure 3.6: Structure of tyro sine (right) and tryptophan (left) and pH-dependence of their reduction potentials. Data are re-plotted from ref. 56 and the pH-dependent solid lines are fit of the data to eq. 3.8 and 3.9.](image-url)
Standard reduction potentials ($E^0$) for the proton coupled oxidation reaction can be calculated from the formal potential at pH 7 and the 59 meV/pH slope (eq 3.8 and 3.9) ($E^0_{\text{TyrOH}^+ / \text{TyrOH}} = 1.34 \text{ V}$ and $E^0_{\text{TrpH}^+ / \text{TrpH}} = 1.43 \text{ V}$).

$$E^0_{\text{TyrOH}^+ / \text{TyrOH}} = E^0_{\text{TyrOH}^+ / \text{TyrOH}} - 0.059pH =$$

$$E^0_{\text{TrpH}^+ / \text{TrpH}} - 0.059(pH-pKa(\text{TyrOH}^+)). (3.8)$$

The reason for the coupling of oxidation with deprotonation is the shift in pKa that is accompanied oxidation. Tyrosine in its reduced form has a pKa of 10 (pKa(TyrOH)) while the oxidised form is very acidic with a pKa of −2 (pKa(TyrOH^+)). In between these pKa-values oxidation causes tyrosine-deprotonation. Outside this pH-range oxidation is no longer coupled to deprotonation, since at high pH tyrosine is already deprotonated and at low pH the tyrosine radical does not deprotonate, and thus the reduction potential is independent on pH. The pH-independent reduction potential at high pH has been determined experimentally to 0.72 V while the low-pH pH-independent potential has to be estimated from the pH-dependent potential at intermediate pH and the pKa of the oxidised tyrosine. This gives a reduction potential for the protonated redox couple of 1.46 V which is above the $[\text{Ru(bpy}_3^3]^-$-reduction potential and tyrosine oxidation is thermodynamically unfavourable thus without the aid of deprotonation. However, with the ester-modified sensitizer discussed in 3.1, tyrosine oxidation without deprotonation becomes slightly exergonic. Tryptophan shows qualitatively the same behaviour as tyrosine but the deprotonated, reduced, form is inaccessible in water solution due to its high pKa (pHa(TrpH) > 14)). The protonated radical on the other hand is readily accessible in water solution and with a pKa(TrpH^+) of 4.7 it is the most stable oxidised form at low pH. The pH-independent potential for the protonated redox couple has been experimentally determined to 1.15 V which is well below the $[\text{Ru(bpy}_3^3]^-$-potential. Quantitatively therefore the tryptophan electron donor differs from the tyrosine donor in that the electron transfer is exergonic both with and without the aid of deprotonation with the $[\text{Ru(bpy}_3^3]^-$ acceptor.

Both tyrosine and tryptophan are colourless compounds and thus they have no visible absorption. The oxidised forms on the other hand absorb in the visible region. Tyrosine radicals give a weak absorption band around 410 nm while the deprotonated tryptophan radical absorbs most strongly at 510 nm. Protonation of the tryptophan radical red-shifts the spectrum to a maximum at 580 nm. Since oxidation of both tyrosine and tryptophan give rise to an absorption change it should be possible to monitor the amino acid
oxidation by transient absorption. Unfortunately in the 410-nm region the [Ru(bpy)$_3$]$_{3^-}$-sensitizer and MV$^{2+}$ give large signals and at 510 and 580 nm viologen radical absorption is strong. However with the complementary [Ru(NH$_3$)$_6$]$_{3^-}$-acceptor the spectral region for tryptophan radical absorption can be opened-up.

3.4 Measuring Electron Transfer Reactions

As stated in the introduction of the chapter time resolved flash photolysis with transient absorption detection has been used to measure the electron transfer rates from the amino acid donor to the [Ru(bpy)$_3$]$_{3^-}$-sensitizer. In this technique a 460-nm laser pulse with a ca 5 ns widths excites the [Ru(bpy)$_3$]$_{2^-}$ chromophore. The laser system used is a frequency doubled and frequency tripled Nd:YAG laser. This laser light at 355 nm passes through an OPO that enables tuning of the excitation wavelength and in this thesis 460 nm excitation light has been used since the [Ru(bpy)$_3$]$_{2^-}$ sensitizer absorbs strongly at this wavelength. To follow the events induced by the laser-flash, light from a pulsed Xe-lamp is passed through the sample at a 90° angle relative to the laser light and is detected as a function of time with a photomultiplier tube. Before entering the photomultiplier the wavelength of the analysing light reaching the detector is selected by a monochromator. By measuring the light as a function of time with and without laser excitation of the sample and subtraction of the two time dependent responses only changes in absorption induced by the laser flash is recorded. With this system it is also possible to measure time resolved emission by blocking the analysing light, thereby determining the emission lifetime of the [Ru(bpy)$_3$]$_{2^-}$ sensitizer.

After laser excitation an electron acceptor quenches the excited sensitizer oxidatively. Figure 3.7 shows the evolution in time of the difference spectrum with the [Ru(bpy)$_3$]$_{3^-}$ sensitizer and [Ru(NH$_3$)$_6$]$_{3^-}$ as acceptor. The ground state/excited state difference spectrum, dominating the transient spectrum at early times after the laser flash, shows a strong negative peak at 450 nm corresponding to a bleach of the MLCT transition. At 370 nm a ligand centred transition without a ground state counterpart, which can also be seen in the free reduced ligand, has appeared. The apparent negative transient absorption above 550 nm is due to excited state emission. While the positive 370-nm peak as well as the emission decays to zero as the excited state disappears the MLCT band decays to a residual negative peak corresponding to the oxidised [Ru(bpy)$_3$]$_{2^-}$ sensitizer. With the experimental conditions used in this case the oxidised sensitizer and the reduced acceptor recombines in a bimolecular reaction since no electron donor to [Ru(bpy)$_3$]$_{3^-}$ other than [Ru(NH$_3$)$_6$]$_{3^-}$ are available. When MV$^{2+}$ is used as acceptor the [Ru(bpy)$_3$]$_{3^-}$/MV$^{2+}$ state shows not only the [Ru(bpy)$_3$]$_{3^-}$/[Ru(bpy)$_3$]$_{2^-}$ bleach
at 450 nm but also strong absorption from the methyl viologen radical at 600 nm and at 400 nm (inset figure 3.7).

Figure 3.7: Evolution in time of the difference spectrum after laser excitation of $[\text{Ru(bpy)}_3]^{2+}$ due to excited state decay through internal relaxation and oxidative quenching by $[\text{Ru(NH}_3)_6]^{3+}$. The apparent bleach at 570-700 nm is due to emission from $[*\text{Ru(bpy)}_3]^{2+}$. The thick-, segmented- and thin lines show the difference spectrum at 30 ns, 170 ns and 850 ns after excitation, respectively. The inset shows the difference spectrum of the final product of oxidative quenching of $[*\text{Ru(bpy)}_3]^{2+}$ by MV$^2^+$, $[\text{Ru(bpy)}_3]^{3+}$ and MV$^+$.

Figure 3.8: (Left) Photo induced electron transfer reactions in Ru(II)(bpy)$_2$(4-Me-4'-(CONH-L-tyrosine ethyl ester)-2,2'-bpy) (Ru(II)-TyrOH). (Right) The $[\text{Ru(bpy)}_3]^{2+}$ sensitizer absorbs light at 460 nm (I) and is oxidatively quenched by MV$^2^+$ (II). Subsequent to quenching the tyrosine part donates an electron to $[\text{Ru(bpy)}_3]^{3+}$ (III). Time resolved transient absorption at 450 nm (thick line) monitoring the instantaneous bleach and recovery of $[\text{Ru(bpy)}_3]^{2+}$ absorption and at 600 nm (thin line) monitoring the build-up and decay of MV$^+$. The faster kinetic of the 450-nm trace compared to the 600-nm trace is due to intramolecular electron transfer from tyrosine to $[\text{Ru(bpy)}_3]^{3+}$. 
In Ru(II)-TyrOH (figure 3.8) a tyrosine donor is covalently linked to the slightly modified \([\text{Ru(bpy)}_3]^{2+}\) sensitizer. Using the flash-quench method described above the Ru(III)-TyrOH/MV\(^{**}\) state is produced. Figure 3.8 (thick lines) shows the recovery of the \([\text{Ru(bpy)}_3]^{2+}\) absorption at 450 nm and the decay of the MV\(^{**}\) absorption at 600 nm (thin line). The fast initial rise of the 600-nm signal corresponds to the quenching process that is resolved on shorter time-scales. From the transients it is clear that the 450-nm signal recovers faster than the 600-nm signal decays indicating a second reaction path, other than recombination, for reduction of \([\text{Ru(bpy)}_3]^{3+}\).

For the reference compound, Ru(II)-Ala (figure 3.9), where the tyrosine unit has been changed to an alanine the 450-nm signal and the 600-nm signal shows the same kinetics and thus only recombination regains the \([\text{Ru(bpy)}_3]^{2+}\) state (figure 3.9). The increased \([\text{Ru(bpy)}_3]^{2+}\) recovery rate in Ru(II)-TyrOH is therefore assigned to intramolecular electron transfer from the tyrosine phenol. In support of this conclusion a small positive absorption at 410 nm from the oxidised tyrosine radical grows in with the same kinetics as the \([\text{Ru(bpy)}_3]^{2+}\) recovery. In neat water the rate constant for intramolecular electron transfer from tyrosine to \([\text{Ru(bpy)}_3]^{3+}\) was determined to \(4 \times 10^4 \text{ s}^{-1}\).

Figure 3.9: (Left) Photo induced electron transfer reactions in Ru(II)(bpy)\(_2\)(4-Me-4’-(CONH-l-alanine ethyl ester)-2,2’-bpy) (Ru(II)-Ala). (Right) As in Ru(II)-TyrOH the \([\text{Ru(bpy)}_3]^{2+}\) sensitizer absorbs light at 460 nm (I) and is oxidatively quenched by MV\(^{**}\) (II). The \([\text{Ru(bpy)}_3]^{2+}\) ground state absorption is recovered by recombination with MV\(^{**}\) hence the recovery rate of the 450 nm signal (thick line) equals the rate of MV\(^{**}\) decay (thin line).
4 Electron Transfer in Solution

In this section a general description of electron transfer reactions in solution is presented. In the first part of the chapter the equations and approximations leading to the semi-classical Marcus equation in the high temperature limit is briefly reviewed starting from a classical description of electron transfer reactions in solution. Also the limitations of this equation are discussed. The following sections highlights the parameters in the Marcus equation that are of importance for the work presented in this thesis.

4.1 Electron Transfer Theory

Electron transfer between free ions or molecules in solution can be divided into three steps: formation of a precursor complex, electron transfer and dissociation of the successor complex. In the first step the electron donor (D) and the electron acceptor (A) diffuse together to form an encounter complex. This is necessary in order to enhance the overlap of the acceptor and donor orbitals and thus the electronic communication between the electron transfer units. In a second step the donor and acceptor reorganises to a nuclear configuration, a Transition State, where electron transfer occurs. Finally, in the third step, the reduced acceptor and the oxidised donor dissociate to form the separated products of the electron transfer reaction.

If electron transfer is very rapid the over-all rate of reaction depends only on the rate limiting diffusion step and no information about the electron transfer reaction can be extracted from the kinetics. Moreover the electron transfer between freely diffusing molecules can occur at many different donor-acceptor configurations and distances and only an average electron transfer rate constant can be determined. To avoid these complications the acceptor and donor can be linked covalently by a separating linker (L) to form a supra molecular D-L-A complex. In this way the diffusion step is avoided, the electron transfer reaction can be studied directly and the length of the bridging unit determines the distance between the donor and the acceptor.

The free energy of the D-L-A complex is a function of many nuclear coordinates, both within the D-L-A complex and in the surrounding solvent, and defines a many-dimensional free energy surface for the Reactant State.
At a certain configuration the system is at a free energy minimum, the equilibrium configuration \((R(D-L-A)^{\text{eq}} = R_R^{\text{eq}})\), which is the most stable nuclear configuration. It is evidently that another nuclear and solvent configuration is more stable in the Product State, \((R(D^+-L-A^-)^{\text{eq}} = R_P^{\text{eq}})\).

The many-dimensional potential energy surfaces in \(D-L-A\) and \(D^+-L-A^-\) can be reduced to a one-dimensional free energy profile along the electron transfer co-ordinate connecting the Reactant State and the Product State (figure 4.1). This free energy profiles constitutes of the vibrational co-ordinates of the reactant that changes its equilibrium values upon electron transfer as well as the solvent orientational and electronic polarization co-ordinates. In this co-ordinate space the free energy profiles along the reaction co-ordinate for the Reactant State and Product State are well approximated with parabolas.\(^{63,64}\)

Figure 4.1: Schematic one dimensional free energy profile for a Reactant State and a Product State along the electron transfer co-ordinate. The difference in energy between the product in its equilibrium position \((R_P^{\text{eq}})\) and the reactant in its equilibrium position \((R_R^{\text{eq}})\) corresponds to the Gibbs free energy of reaction \((\Delta G^0)\). Classically crossing from the reactant surface to the product surface may occur only at the transition state (TS) and the energy required to bring the reactant from \(R_R^{\text{eq}}\) to TS is the activation energy \((\Delta G^0)\). The reorganisation energy \((\lambda)\) is defined as the energy required to distort the reactants from \(R_R^{\text{eq}}\) to \(R_P^{\text{eq}}\) without transferring an electron.

Compared to the nuclei the electron being transferred is a light particle and the Frank-Condon principle can be applied for the electron transfer reaction. That means that the nucleus does not move or change their momentum significantly during the electron motion. Electron transfer then has to occur close to where the reactant and product surfaces cross, the electron transfer transition state (TS). At this position on the free energy surface the reactant can cross over to the Product State without changing the
nuclear co-ordinates and also without changing the direction of the nuclei considerably. In order for reaction to occur the Reactant State thus has to distort from the equilibrium position to the Transition State. The energy required for that distortion is the activation energy, $\Delta G_0^\circ$. Defining the reorganisation energy ($\lambda$) as the energy required to bring the Reactant State from its equilibrium position to the product equilibrium position without transferring an electron, the activation energy is given by eq 4.1, where $\Delta G^0$ is the difference in equilibrium energy for the products and the reactants. This result is simply an effect of the parabolic shape of the free energy surfaces along the reaction co-ordinate.

$$\Delta G_0^\circ = \frac{(\Delta G^0 + \lambda)^2}{4\lambda} \quad (4.1)$$

According to classical transition state theory the rate constant for electron transfer is given by the frequency of passage through the transition state ($v_n$), the electronic transmission coefficient ($\kappa_{el}$) and the fraction of molecules at the transition state (eq 4.2).

$$k_{ET} = v_n \kappa_{el} e^{-\frac{\Delta G_0^\circ}{k_B T}} \quad (4.2)$$

Figure 4.2: In non-adiabatic electron transfer (left) the system stays on the reactant surface on most transitions through the Transition State and only occasionally does the system cross from the reactant surface to the product surface. For adiabatic electron transfer reactions (left) on the other hand the system always moves along the lower energy surface.

The exponential factor is the fraction of reactant molecules at the Transition State given by the Boltzmann distribution. The transmission coefficient defines two different regimes of electron transfer reactions, adiabatic and non-adiabatic. If $\kappa_{el}$ is close to unity the reaction is adiabatic and the Reactant State transfers to the Product State on every passage through the transition state. This is illustrated in figure 4.2. On the other hand if the
reactant proceeds through the Transition State on the reactant surface and only occasionally crosses over to the Product surface the reaction is non-adiabatic, illustrated in fig 4.2, and the transmission coefficient is much less than unity. What determines whether an electron transfer reaction is in the adiabatic or non-adiabatic regime is the electronic coupling between the electron acceptor and the electron donor and a more involved quantum mechanical treatment is necessary.

Starting from the Golden Rule expression for the transition rate constant from one initially prepared Reactant State, i, to a manifold of Product States, j, eq 4.3. The Golden rule expression is based on perturbation theory. It is thus valid only for small perturbations of the Reactant State by the Product State, and effectively valid only for non-adiabatic electron transfer reactions. Moreover the electron transfer is assumed to be irreversible which is met also if the coupling between reactant and product is small and the initially produced vibrational state in the Product State relax rapidly to other vibrational states.

\[ k_{i\rightarrow j} = \frac{2\pi}{\hbar} H_{rp}^2 \sum_j \left\langle \rho_R \right| \delta(\varepsilon_R - \varepsilon_P) \left| \rho_P \right\rangle \]  

(4.3)

In this equation \( \rho_R \) and \( \rho_P \) are the vibrational wave functions for state \( j \) and \( i \), respectively and \( H_{rp} \) is the electronic coupling energy between the Reactant State and the Product State. The delta function ensures conservation of energy during the transition. In order to reach the transition rate constant, for the transition from the Product State to the Reactant State, transitions from all reactant vibrational states has to be taken into account. The sum of the transition rates from all vibrational states in the Reactant State to the Product State has to be determined and weight by the fraction of molecules in that specific vibrational state at all times. If the coupling to the solvent is strong so that equilibrium between the solvent and the molecule is reached fast compared to the electron transfer rate the fraction of molecules in a specific vibrational state is given by the Boltzmann distribution, eq 4.4, at all times during the electron transfer reaction. Rapid vibrational relaxation in the Product State also ensures the second requirement for the Golden Rule treatment. Thus the electron transfer rate constant is given by eq 4.5.

\[ P_R = e^{-\Delta_{RT}/kT} \sum_i e^{-\Delta_{RT}/kT} \]  

(4.4)

\[ k_{R\rightarrow P} = \frac{2\pi}{\hbar} H_{rp}^2 \sum_i \sum_j \left\langle \rho_P \right| \delta(\varepsilon_P - \varepsilon_R) \left| \rho_R \right\rangle \]  

(4.5)
The sum in eq 4.5 constitutes of all vibrational modes, both internal and solvent modes. However in most cases the low frequency solvent modes can be treated classically and the internal vibrational modes can be approximately replaced by an average mode frequency. Furthermore at room temperature the separation between the vibrational levels, given by the average mode frequency, is often much less than the thermal energy and the vibrational dynamics can be treated within the framework of classical physics. The result of these approximations is called the semi-classical Marcus equation in the high temperature limit, eq 4.6.

$$k_{R \rightarrow P} = \frac{2\pi}{\hbar \sqrt{4\pi k_B T \lambda}} e^{\frac{\mu G^0 + \lambda^2}{4\hbar \sqrt{T \lambda}}}$$

(4.6)

4.2 The Reorganisation Energy

The reorganisation energy is a measure of the structural difference in the equilibrium configuration between the Reactant and Product State as well as the difference in solvent orientation and polarisation. In the Marcus treatment of electron transfer reactions the reorganisation energy can be viewed as a horizontal displacement of the product free energy surface relative to the reactant free energy surface (figure 4.3). Such a distortion increases the activation energy and thus decreases the electron transfer rate constant in the Marcus normal region (see below).

Solvent contributions to the reorganisation energy, \(\lambda_{\text{out}}\), are often separated from the internal reorganisation energy, \(\lambda_{\text{in}}\), that depends on the structural differences, differences in bond lengths, in the equilibrium configurations (eq 4.7). For harmonic vibrations the inner reorganisation energy is given by eq 4.8, where \(r^0_R\) and \(r^0_P\) are the equilibrium bond lengths in the reactant and Product State respectively and \(f_i\) is the reduced force constant for the \(i\)th vibration.

$$\lambda = \lambda_{\text{in}} + \lambda_{\text{out}}$$

(4.7)

$$\lambda_{\text{in}} = \frac{1}{2} \sum_i f_i (r^0_R - r^0_P)^2$$

(4.8)
Figure 4.3: An increased reorganisation energy, from $\lambda_1$ to $\lambda_2$, corresponds to a horizontal distortion of the product free energy surface relative to the reactant surface, i.e. compare Product State solid line with Product State dotted line. In the Marcus normal region (see chapter 4.4) this gives a higher activation energy and a lower electron transfer rate constant.

In the continuum model for a one electron transfer treating the solvent as a dielectric and assuming spherical reactant molecules the outer reorganisation energy is given by eq 4.9, where $a_A$ and $a_D$ are the radius of the electron acceptor and the electron donor respectively and $r_{DA}$ is their center-to-center distance. The larger the distance the electron is transferred the higher the outer reorganisation energy. Also the polarity of the solvent affects the outer reorganisation energy. For non-polar solvents ($\varepsilon_{op} = \varepsilon_s$) the outer reorganisation energy is zero and increases to higher values as the solvent becomes more polar.

$$\lambda_{out} = \frac{e^2}{4\pi\varepsilon_0} \left( \frac{1}{2a_D} + \frac{1}{2a_R} - \frac{1}{r_{DA}} \right) \left( \frac{1}{\varepsilon_{op}} - \frac{1}{\varepsilon_s} \right)$$ (4.9)

4.3 The Electronic Coupling Energy

The electronic coupling energy depends on the orbital overlap between the electron donor and the electron acceptor. Consequently the overlap depends on the distance between the electron transfer units. At sufficiently large distances from the nucleus any orbital decreases exponentially with distance and so does the electronic coupling energy. Hence, the electron transfer rate constant decreases exponentially with donor-acceptor distance at sufficiently large donor-acceptor distances (eq.4.10).

$$k = k_0 e^{-\beta(r-r_0)}$$ (4.10)
Here \( k_0 \) is the electron transfer rate constant at a centre to centre distance limited by radius of the donor and the acceptor \( (r_0) \), \( r \) is the donor acceptor distance and \( \beta \) determines the decrease of electronic coupling with distance.

In vacuum the distance decay constant, \( \beta \), is expected to be ca. 3.4 Å\(^{-1}\) and electron transfer is limited to rather short distances.\(^71\) However a mediating bridge can significantly enhance long range electron transfer rates between the electron donor and the electron acceptor.\(^72,62\) This bridge mediated electron transfer can occur in either of two ways, by trivial sequential electron transfer to the bridge or by a super exchange mechanism\(^73,74\) where the electron at no time resides on the bridging units. In the super exchange mechanism instead the function of the mediating bridge is to enhance the electronic coupling of the electron transfer species by coupling of either its LUMO orbitals (electron transfer) or its HOMO orbitals (hole transfer) to the electron donor-acceptor orbitals.

### 4.4 The Gibbs Free Energy of Reaction

In general an electron transfer reaction is simply a redox process where an electron donor is oxidised by the electron acceptor (scheme 4.11). Usually such redox reactions can be divided into cell schemes with specific standard potentials (scheme 4.12 and 4.13). (By convention standard potentials are given as reduction potentials with standard hydrogen electrode (SHE) as reference).

\[
D + A \rightarrow D^+ + A^- \quad E^0 = E_A^0 - E_D^0 \tag{4.11}
\]

\[
D^+ + e^- \rightarrow D \quad E_D^0 \tag{4.12}
\]

\[
A + e^- \rightarrow A^- \quad E_A^0 \tag{4.13}
\]

The difference in reduction potential for the electron acceptor \( (E_A^0) \) and the electron donor \( (E_D^0) \) then give the standard potential for the overall reaction \( (E^0) \). Standard potentials are related to the standard Gibbs free energy of reaction by eq 4.14, where \( F \) is the Faraday constant and \( n \) is the number of charges transferred in the reaction. For one-electron transfer reactions \( n \) is one. Reaction driving forces \( (-\Delta G^0) \) for electron transfer reactions can thus be determined if the reduction potential for the individual electron transfer units are known.

When the reactants and/or the products are charged and in close contact, the coulombic interaction between the donor and the acceptor has to be taken into account. These work terms corresponds to the coulombic work required to bring the reactants together \( (w_R) \) and to bring the products
together \((w_P)\) and are given by equation 4.15 and 4.16 respectively. In eq. 4.15 and 4.16 \(r_{DA}\) is the donor acceptor distance, \(\varepsilon^0\) the permeability of vacuum, \(\varepsilon_s\) the dielectric constant of the solvent, \(z_D^R\) and \(z_A^R\) is the charge of the donor and acceptor in the Reactant State and \(z_D^P\) and \(z_A^P\) is the charge of the donor and acceptor in the Product State.

\[
\Delta G^0 = -nFE^0 = -nF(E_A^0 - E_D^0) - w_R + w_P \\
\text{(4.14)}
\]

\[
w_R = \frac{z_D^R z_A^R}{r_{DA}\varepsilon^0} \\
\text{(4.15)}
\]

\[
w_R = \frac{z_D^P z_A^P}{r_{DA}\varepsilon^0} \\
\text{(4.16)}
\]

The driving force is the difference in free energy for the reactant and product equilibrium states. In the Marcus treatment this corresponds to a vertical displacement of the Product State free energy surface relative to the reactant free energy surface (figure 4.4). When the driving force equals the reorganisation energy the exponential factor in eq 4.6 vanishes, i.e. the reaction is non-activated and the electron transfer rate constant is at maximum.

\[
\text{Figure 4.4: An increased driving force, from } -\Delta G_1^0 \text{ to } -\Delta G_2^0, \text{ corresponds to a vertical distortion of the product free energy surface relative to the reactant surface, i.e. compare Product State solid line with Product State dotted line. In the Marcus normal region this gives a lower activation energy and a higher electron transfer rate constant.}
\]

A lower and higher driving forces the electron transfer reaction becomes activated and the electron transfer rate constant decreases (Figure 4.5). In the driving force region where \(-\Delta G^0 < \lambda\) the electron transfer rate constant increases with driving force; This is called the Marcus normal region. At
large driving forces ($-\Delta G^0 > \lambda$) the rate constant instead decreases with increased driving force. This inverted region has been verified experimentally in linked donor acceptor systems.

![Figure 4.5: Marcus parabola showing the electron transfer rate constant as a function of driving force ($-\Delta G^0$). In the normal region ($-\Delta G^0 < \lambda$) the electron transfer rate constant increases with driving force due to a decreased activation energy. When ($-\Delta G^0 = \lambda$) the reaction occurs without activation. At even higher $-\Delta G^0$, in the inverted region ($-\Delta G^0 < \lambda$), the reaction again requires activation and the rate constant decreases with driving force. The insets show schematically the free energy profile along the reaction co-ordinate in the different regions for the Reactant State (black surfaces) and the Product State (grey surfaces).

4.4.1 Special Case of Photo-Induced Electron Transfer Reactions

An excited molecule is always a stronger oxidant as well as a stronger reductant compared to its ground state counterpart. This is because excitation promotes an electron to a high energy orbital leaving a low energy orbital half empty. The extra energy gained, corresponding to the excitation energy ($E_h$), can be used for reduction. Since the electron rapidly, before it has time to react, relaxes to the vibrational ground state in condensed phases the extra energy for the excited electron corresponds to the lowest vibrational state excitation energy ($E_{00}$). Hence the reduction potential for oxidation of the excited molecule is given by eq 4.17. For reduction of an excited molecule the orbital involved is the half-empty HOMO. The energy for this orbital is lower in energy compared to the LUMO in the ground state by $E_{00}$. Hence, for reduction of an excited molecule the reduction potential is given by eq 4.18.

\[
E_{*D} = E_D - E_{00} \quad (4.17)
\]

\[
E_{*A} = E_A + E_{00} \quad (4.18)
\]
In this chapter the semi-classical Marcus equation in the high temperature limit is extended to include the coupling of electron transfer to proton motions. As stated in chapter 3.3 oxidation of tyrosine is coupled to deprotonation of the phenolic group in water solution at most pH. The electron transfer from tyrosine to [Ru(bpy)$_3$]$^{3+}$ in Ru(II)-TyrOH depicted in figure 3.8 is thus coupled to deprotonation (figure 5.1). This supra molecular system will serve as base for the discussion of this type of proton coupled electron transfer (PCET) reactions where the electron and proton moves in different directions, bi-directional PCET reactions, but the discussion is generally applicable to bi-directional PCET in other systems as well. The other type of PCET, unidirectional PCET where the electron and proton moves from the same molecular unit to the same acceptor, has been extensively studied by other groups both theoretically and experimentally.

The first part of the chapter, 5.1-5.3, comprises a general discussion of the different possible mechanisms for PCET, including kinetic limitations (5.1), energetics (5.2) and predictions from general electron transfer theory (5.3), with Ru(II)-TyrOH as basis for discussion. In 5.4 experimental tools (pH- and temperature dependence of the PCET rate constant as well as the deuterium isotope effect) for mechanistic discrimination is presented.
Finally, in the last part of the chapter I present what kinetic parameters that control the outcome of the competition between the different mechanisms and how to selectively favour one reaction path by modifying the electron accepting unit ([Ru(bpy)₃]³⁺) or the electron donor unit (TyrOH).

5.1 Mechanisms of Proton Coupled Electron Transfer

The electron transfer from tyrosine to [Ru(bpy)₃]³⁺ gives the oxidised tyrosine radical (TyrOH⁺). In neutral water solution this radical will deprotonate rapidly due to the low pKa-value of the oxidised tyrosine (pKa(TyrOH⁺) = -2) (figure 5.2). With a diffusion controlled rate of protonation, \( k_p = 1 \times 10^{11} \text{ M}^{-1}\text{s}^{-1} \), the rate constant for deprotonation is \( k_d = k_p \times 10^{p\text{Ka}(\text{TyrOH(ox)})} = 1 \times 10^{13} \text{ s}^{-1} \), considering deprotonation to H₂O only. Deprotonation of the oxidised tyrosine is thus much faster than the experimentally found rate constant for the over-all reaction, \( k = 4 \times 10^4 \text{ s}^{-1} \) (see chapter 3) and the primary electron transfer step limits the over-all reaction rate.

Figure 5.2: Possible mechanisms for PCET in Ru(II)-TyrOH together with \( \Delta G^0 \) for the ET steps and the pKa-values for deprotonation reactions: A pure electron transfer (ET) yields the protonated tyrosine radical (TyrOH⁺) that rapidly deprotonates (PT⁺) to form (TyrO⁻). A primary deprotonation (PT) of tyrosine gives the tyrosinate ion (TyrO⁻). Electron from the tyrosinate then gives the final product. In the CEP reaction electron transfer and deprotonation occurs concerted in one single reaction event.

An alternative route from the Reactant State to the Product State is an initial deprotonation of the reduced tyrosine followed by electron transfer from the tyrosinate anion to [Ru(bpy)₃]³⁺ (PTET⁺) (figure 5.2). The rate constant for deprotonation of tyrosine to water can be determined from the pKa-value of the reduced tyrosine and the diffusion controlled rate constant for protonation as above, \( k_d = k_p \times 10^{p\text{Ka}(\text{TyrOH(red)})} = 10 \text{ s}^{-1} \). At high pH the concentration of hydroxide ions becomes sufficiently large that
deprotonation to OH may be significant. An upper limit for the corresponding rate constant can be approximately determined, assuming a non-activated reaction, from the diffusion controlled rate constant for deprotonation, \( k_d^{OH} = 1 \times 10^{10} \text{ M}^{-1}\text{s}^{-1} \) at any hydroxide concentration. The over-all rate constant for the deprotonation of tyrosine, given by the sum of the two contributions, shows a strong pH-dependence at high pH (figure 5.3) and decreases to the asymptotic value of the deprotonation rate constant for deprotonation to water at neutral pH. This rate constant however is too low to be consistent with the observed rate constant at neutral pH found experimentally (see chapter 3). Although this mechanism is ruled out in the Ru(II)-TyrOH system it will be discussed as an option in the general case.

![Figure 5.3: pH-dependence of deprotonation rate for tyrosine. The dotted and dashed lines represent deprotonation to water and deprotonation by OH respectively. The solid line is the sum of the two contributions to the deprotonation rate.](image)

In addition to the two sequential mechanisms described above, electron transfer followed by deprotonation (ETPT') and deprotonation followed by electron transfer (PTET'), for the PCET depicted in figure 5.1 a concerted electron transfer/deprotonation mechanism is possible (CEP). In this mechanism a single reaction event comprises both electron transfer and deprotonation (figure 5.2). It is therefore not kinetically limited by any of the elementary reactions, electron transfer or deprotonation, but instead by the co-operative motion of the electron and the proton. Due to the complexity of the reaction this mechanism is often considered as an improbable event. In this work however I present data that shows that the concerted mechanism is the dominant reaction path for tyrosine oxidation in water solution under most conditions employed.
5.2 Energetics of Proton Coupled Electron Transfer

For the three possible reaction mechanisms in the previous section there are four stable to consider, Ru(III)-TyrOH (I), Ru(II)-TyrOH\(\text{II}^+\) (II), Ru(III)-TyrO\(\text{II}^-\) (III) and Ru(II)-TyrO\(\text{II}^-\) (IV). Although the intermediate states may be short-lived they correspond to local free energy minima and will be considered as states. The free energy change going from the reactant-state to any of the other three states can be determined from the reduction potential for the [Ru(bpy)\(\text{III}\)]\(^{3+}\)/[Ru(bpy)\(\text{II}\)]\(^{2+}\) couple and the relevant tyrosine reduction potential (figure 3.6 and table 5.1) according to the procedure in 4.4.

Table 1.1: Standard reduction potentials vs. SHE for [Ru(bpy)\(\text{III}\)]\(^{3+}\) and for tyrosine in different possible protonation states in water solution.

<table>
<thead>
<tr>
<th>Redox couple</th>
<th>Ru(^{3+})/Ru(^{2+})</th>
<th>TyrOH(^{\text{II}+})/TyrOH</th>
<th>TyrO(^{\text{II}+}) + H(^+)/TyrOH</th>
<th>TyrO(^{\text{II}+})/TyrO(^{\text{II}^-})</th>
</tr>
</thead>
<tbody>
<tr>
<td>E(^0) (V)</td>
<td>1.26</td>
<td>1.46</td>
<td>1.34</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Consider the pure electron transfer reaction in the ETPT’ mechanism from state I to state II (eq 5.3). The reaction standard free energy, \(\Delta G^0\), is determined from the relevant standard reduction potentials (eq 5.4). Because both the oxidised and the reduced forms are protonated, the tyrosine potential to be used is the pH-independent potential at pH below the pKa of the oxidised tyrosine. This potential is relevant at any pH for the ET-reaction from I to II.\(^8\)

\[
[Ru(bpy)\text{I}]^{3+} + e^- \rightarrow [Ru(bpy)\text{II}]^{2+} \quad (5.1)
\]

\[
\text{TyrOH}^{\text{II}+} + e^- \rightarrow \text{TyrOH} \quad (5.2)
\]

\[
[Ru(bpy)\text{I}]^{3+} + \text{TyrOH} \rightarrow [Ru(bpy)\text{II}]^{2+} + \text{TyrOH}^{\text{II}+} \quad (5.3)
\]

\[
\Delta G^0 (ET) = -(E^0_{\text{Ru}^{3+}/\text{Ru}^{2+}} - E^0_{\text{TyrOH}^{\text{II}+}/\text{TyrOH}}) \quad (5.4)
\]

The reaction from state I to state III is a deprotonation of tyrosine and we only have to consider the tyrosine part of the molecule (eq 5.5). For the dissociation reaction in eq 5.5 the standard Gibbs free energy of reaction is given by eq 5.6, where Ka is the dissociation constant for tyrosine and R is the gas constant. This free energy is relevant at standard conditions, i.e. at pH 0. At other pH values the formal Gibbs free energy of reaction, \(\Delta G^0\), has to be used (eq 5.7).

\[
\text{TyrOH} \rightarrow \text{TyrO}^- + H^+ \quad (5.5)
\]

\[
\Delta G^0 (\text{ET}) = -(E^0_{\text{Ru}^{3+}/\text{Ru}^{2+}} - E^0_{\text{TyrOH}^{\text{II}+}/\text{TyrOH}}) \quad (5.6)
\]

\[
\Delta G^0 (\text{Diss}) = -(RT \ln K_a) \quad (5.7)
\]
\[
\Delta G^0 = -RT \ln K_a(TyrOH) = \ln 10RTpK_a(TyrOH) 
\]

\[\Delta G^{0'} = \ln 10RT(pK_a(TyrOH) - pH)\]  

(5.6)

(5.7)

In the concerted electron transfer/deprotonation reaction from state I to state IV (eq. 5.10) the reaction can be divided into two redox reactions (eq. 5.8 and 5.9) as in the pure ET reaction discussed above. As in the ET reaction the Ruthenium part is reduced from Ru(III) to Ru(II). The concomitant tyrosine oxidation however is different from the pure ET case; the oxidation is coupled to deprotonation of the tyrosine. The formal redox potential for this reaction decreases linearly with pH, with a slope of -59 meV per pH unit, (figure 3.6) and we expect the formal reaction free energy to be pH-dependent (eq 5.11).

\[
[Ru(bpy)_3]^{3+} + e^- \rightarrow [Ru(bpy)_3]^{2+} \]  

(5.8)

\[
\text{TyrO}^* + H^+ + e^- \rightarrow \text{TyrOH} 
\]

\[E^0_{\text{TyrO}^* + H^+ / \text{TyrOH}} = E^0_{\text{TyrOH}^{**} / \text{TyrOH}} - 0.059(pH-pK_a(TyrOH^{**})) \text{ V} \]  

(5.9)

\[
[Ru(bpy)_3]^{3+} + \text{TyrOH} \rightarrow [Ru(bpy)_3]^{2+} + \text{TyrO}^* + H^+ \]  

(5.10)

\[E^0_{\text{TyrO}^* / \text{TyrOH}^{**}} = E^0_{\text{TyrOH}^{**} / \text{TyrOH}} + 0.059(pH-pK_a(TyrOH^{**})) \]  

(5.11)

Since the Reactant State and the final state are the same, irrespective of mechanism, the energetics for any of the secondary steps, ET’ and PT’ (figure 5.2), can be determined from the difference in \(\Delta G^0\) for the CEP reaction and the relevant primary step in the sequential reactions. Alternatively the driving force can be calculated directly using the same line of argument as above (eq 5.12 and 5.13).

\[\Delta G^0(CEP) = -(E^0_{Ru^{3+}/Ru^{2+}} - E^0_{\text{TyrOH}^{**} / \text{TyrOH}} + 0.059(pH-pK_a(TyrOH^{**})) \]  

(5.12)

\[\Delta G^{0'}(ET') = -(E^0_{Ru^{3+}/Ru^{2+}} - E^0_{\text{TyrO}^* / \text{TyrO}^-}) \]  

(5.13)

\[\Delta G^{0'}(PT') = \ln 10RT(pK_a(TyrOH^{**}) - pH) \]  

(5.14)

In summary of this chapter, coupling of the electron motion with a deprotonation can occur only at pH-values where the tyrosine is initially protonated and where the tyrosine radical deprotonates, that is pKa(TyrOH^{**}) = -2 < pH < pKa(TyrOH) = 10. In this pH range deprotonation of TyrOH is energetically unfavourable and deprotonation of TyrOH^{**} is favoured. In order to close the thermodynamic cycle in figure 5.2 it is evident that the driving force for ET will be smaller than the driving
force for CEP and that the ET’ reaction will be the most energetically
favoured of the three electron transfer reactions in any PCET system. Also
for the secondary step to occur it has to be exergonic. That means that the
primary steps in the step-wise mechanisms have lower driving forces
compared to the concerted reaction. In general therefore the CEP reaction
must be considered as an energy conservative mechanism in that all energy
available is used in the rate-determining step and not wasted in secondary
reactions.

5.3 Kinetics of Proton Coupled Electron Transfer

In the Marcus theory for non-adiabatic electron transfer reactions, discussed
in chapter 4.1, the many-dimensional potential energy surfaces for the
Reactant State and the Product State were reduced to a one-dimensional free
energy profile along the electron transfer co-ordinate connecting the
Reactant State and the Product State. For PCET reactions it is useful to
separate the reaction co-ordinate into nuclear motions associated with pure
electron transfer and nuclear motions associated with pure proton
transfer.85,86 Figure 5.4 shows a schematic free energy landscape for the
Ru-TyrOH system where the proton- and electron transfer co-ordinates have
been divided into separate parts. In this figure the ETPT’ reaction (red
arrows) consists of nuclear distortions along the electron transfer co-ordinate
giving Ru(II)-TyrOH\textsuperscript{+} followed by distortions now along the proton
transfer co-ordinate to form the final product Ru(II)-TyrO\textsuperscript{−}. The PTET’
reaction (green arrows) instead starts with distortions along the proton
transfer co-ordinate, giving Ru(III)-TyrO\textsuperscript{−}, that is followed by electron
transfer co-ordinate distortions to form the final product. In the CEP reaction
(black arrow) the nuclear distortions comprise simultaneous motion along
the electron transfer co-ordinates and along proton transfer co-ordinates.

In paper I and III we argue that the distortion for the CEP reaction is
larger than for any of the primary, rate determining, steps in the sequential
mechanisms. Following the discussion in 4.2 a larger distortion, a larger
distance between equilibrium position of the Reactant State and Product
State, gives a higher reorganisation energy. This can also be deduced by
inspection of eq 4.8 and eq 4.9. In eq 4.8 the inner reorganisation energy for
the electron transfer is given by the difference in bond lengths in the
Reactant State and in the Product State. In the CEP mechanism the tyrosine
phenolic OH-bond is broken and the equilibrium bond distance is markedly
changed.87 In the ET-reaction on the other hand the phenolic proton is still
attached to tyrosine and thus the change in bond length is less. Also the outer
reorganisation energy is increased in CEP compared to the ET reaction.
Since the released proton carries a positive charge the effective charge
separation is larger in CEP than in ET and thus the outer reorganisation
energy is higher. The larger distance the proton is transferred in CEP also requires larger reorganisations in the solvating shell around the proton. We therefore expect the concerted reaction to have, intrinsically, a higher reorganisation energy compared to the sequential reaction mechanisms. In paper III this was verified experimentally (see section 5.5).

![Figure 5.4: Schematic free energy surface for proton coupled electron transfer reactions with separate co-ordinates for nuclear motions associated with electron transfer and deprotonation. In an ETPT' mechanism (red arrows) the reactants first distort along the electron transfer co-ordinate and then along the proton transfer co-ordinate. The green arrows follow the PTET' reaction path with a primary proton co-ordinate distortion followed by an electron transfer co-ordinate distortion. In the CEP reaction (black arrow) the systems moves simultaneous along both the proton transfer co-ordinate and the electron transfer co-ordinate. This gives intrinsically a high reorganisation energy for the CEP. Inset: The energy of the final state decreases as pH increases. This decreases the activation barrier for the CEP reaction (black surfaces) and hence the rate constant for CEP is pH-dependent. For pure ET on the other hand the reaction is independent on pH (red surface).](image)

In the Marcus normal region, the concerted reaction is thus unable to compete with the sequential mechanisms for isoenergetic primary reactions. However we saw in the last chapter that the driving force for CEP is larger than for the ET or the PT step and the relative importance of increased driving force and larger reorganisations for CEP governs the choice of mechanism in PCET reactions (paper III and V).
5.4 pH-Dependence of Proton Coupled Electron Transfer: An Experimental Tool for Determining the Mechanism

In the Ru(II)-TyrOH complex that served as basis for the discussion above we found that the rate constant for electron transfer from tyrosine to photo-oxidised ruthenium-tris-bipyridine was strongly dependent on pH (figure 5.5) (paper I). In the pH-region where the electron transfer is coupled to deprotonation of the tyrosine moiety, pKa(TyrOH\textsuperscript{\textsuperscript{\textsuperscript{+}}}) > pH > pKa(TyrOH), the rate constant increases monotonously with pH. Above pKa(TyrOH), where the tyrosine is initially deprotonated, the rate constant is much higher, k\textsubscript{ET'} = 5\times10^7 \text{ s}^{-1}, and independent of pH. This high pH region thus correspond to the ET' reaction in figure 5.2. In line with the statement that the deprotonation reaction limits the overall rate for the PTET' reaction path the ET' rate constant is much higher than the PCET rate constant.

Figure 5.5: pH-dependence of the electron transfer rate constant for intramolecular electron transfer between tyrosine and photo-generated [Ru(bpy)\textsubscript{3}]\textsuperscript{3+} in Ru(II)-TyrOH. In the low pH-region the electron transfer rate constant increases monotonously with pH indicative of a CEP mechanism. The solid line is a theoretical function for the pH-dependence (see text). Above the tyrosine pKa the rate constant is much higher and independent of pH. Around the pKa the two phases co-exists. The dotted line is a theoretical pH-dependence of the rate constant for deprotonation of tyrosine (See section 5.1).

In the vicinity of pKa(TyrOH) the electron transfer kinetics is biphasic. This double exponential behaviour could be explained by any of the PCET mechanisms. For PTET' however this requires that the electron transfer is much faster than to the deprotonation reaction. Otherwise the pH-dependence of the over-all rate constant would show a titration behaviour, with a rate constant given by the fraction of tyrosinates times the electron
transfer rate constant, with an inflection point at pH = pKa(TyrOH) and no biexponential kinetics would be observed (solid line figure 5.6).

![Figure 5.6: Expected pH-dependence of the rate constant for PTET' limited by electron transfer (solid line) and for ETPT' (dotted line) limited by electron transfer.](image)

The pH dependence of the electron transfer rate constants in the PCET pH-region can be used to discriminate between the different mechanisms. (1) In the ETPT' reaction sequence electron transfer limits the reaction rate as stated previously. This reaction does not involve any proton transfer steps and is thus independent of pH and pH-independent rate constants are expected (dotted line figure 5.6). (2) A proton transfer first mechanism is rate limited by the deprotonation rate and the pH-dependence of the over-all rate constant would have the same strong pH-dependence as the deprotonation rate discussed in 5.1 (solid line figure 5.3). Evidently this pH dependence is much stronger than the observed pH-dependence in Ru(II)-TyrOH (Figure 5.5). Also the buffer system used may act as a primary proton acceptor. However the rate constant was not noticeably changed from neat water to a buffer concentration of 0.01 M at pH 7. A primary deprotonation of tyrosine by the buffer base would also give the same strong pH-dependence as deprotonation by OH⁻ since the concentration of the deprotonated base increases exponentially with pH. Involvement of the buffer system in the PCET reaction can therefore also be excluded under the conditions employed. (3) The remaining CEP-mechanism thus has to account for the observed pH-dependence.

In section 5.2 we found that the reaction driving force for CEP increases by 0.059 eV per pH unit due to the increased mixing entropy of the released proton, eq 5.11. Using this pH-dependent ΔG° in the Marcus equation eq 4.6 we were able to reproduce the observed pH-dependence of the PCET rate constant (solid line figure 5.5) (paper I). In this fit the reorganisation energy was fixed to the reorganisation energy independently determined by temperature measurements (see section 5.5). This result however is highly surprising (see for example ref. 84) since none of the elementary reactions
(ET or PT) constituting the CEP depends kinetically on a pH-dependent driving force. The PT reaction has exactly the same pH-dependence of the formal potential as the CEP but the rate constant for deprotonation to water is independent of pH. At pH-values where deprotonation to OH\(^-\) dominates the deprotonation rate increases with ten times per pH unit due to an increased hydroxide concentration (figure 5.3). The second order rate constant \(k_{d, OH}^-\) however depends on the nature of the acid and is independent of pH. A pH-independence in the low pH-regime can be understood considering the high concentration of water molecules compared to OH\(^-\) surrounding the phenolic proton. The proton accepting properties of water determines the rate of deprotonation since a water molecule or a small water cluster acts as primary proton acceptor irrespective of pH. This gives a rate that is independent of pH, although the overall reaction (with deprotonation to the bulk) has a pH-dependent driving force. In contrast the CEP reaction rate depends on pH and hence on the proton activity in the solvent, which is a macroscopic property. As the pH increases the proton chemical potential decreases as illustrated by the inset in figure 5.4. This decrease in potential apparently affects the activation energy for the CEP reaction. The difference in behaviour between a pure deprotonation reaction and a deprotonation reaction concertedly coupled to electron transfer is not understood. However the pH-dependence data clearly shows such a qualitative difference and this is the most important result of paper I.

If a parabolic shape of the potential surface along the proton transfer co-ordinate is assumed in the region close to the transition state the dependence of the rate constant on pH in CEP can be described using a pH-dependent driving force in the Marcus equation as above (inset figure 5.4). This gave an excellent agreement with the data (see figure 5.5). In this treatment the transferring proton is considered as a classical particle. With a vibrational energy for the phenolic tyrosine proton of about 3600 cm\(^{-1}\) it is evident that the thermal energy, k\(_B\)T, is insufficient to reach the high temperature limit of the semi-classical Marcus equation, eq. 4.6, since k\(_B\)T \(\ll\) h\(\nu\). A classical treatment of the proton motion is therefore an oversimplification. However, in ref. 85 multi-state continuum theory was applied to model the PCET reaction in Ru(II)-TyrOH. In this model a single H\(_2\)O molecule was the proton acceptor. Although a pH-dependent \(\Delta G^0\) is not consistent with this model the authors used our experimental pH-dependent \(\Delta G^0\) values as input parameters to reproduce the observed pH-dependence of the CEP rate constants. The agreement was good, which supports our earlier interpretation that the pH-dependent \(\Delta G^0\) can be used to qualitatively explain the pH-dependent rate. The calculations were also able to quantitatively reproduce the experimentally found temperature dependence (see below) for the CEP reaction and for the ET' reaction as well as their relative rates. Since both models gives comparable results we believe that the classical treatment of the proton motion is a good
compromise between theoretical accuracy and experimental control and comprehensibility of the variables.

Further support for a concerted electron transfer/deprotonation reaction was found by replacing the water solvent by deuterium dioxide (Paper V). Since the phenolic tyrosine proton involved in the PCET is exchangeable the phenol is deuterated by the solvent change. Deuteration of the phenol significantly reduced the PCET rate constant by a factor of 2-2.5 indicating that proton motions are in fact associated with the electron transfer reaction. The reduction in rate by deuteration is due to the narrower probability densities for deuterium that decreases the overlap between the initial and final proton/deuteron states.85,92 Opposing this effect the deuterium states are lower in energy compared to the proton states making them thermally more accessible.92 A similar deuterium-isotope effect (k_H/k_D = 3) has been calculated in reference 85 for the Ru(II)-TyrOH system using the quantum mechanical model discussed above. Also Cukier calculated a similar deuterium isotope effect for the same type of reactions.92

![Figure 5.7: Simulation of a Marcus parabola for the CEP reaction from tyrosine to [Ru(bpy)_3]^{3+} (solid line) in Ru(II)-TyrOH together with the data presented in figure 5.5 (open circles).](image)

To summarise this part, we have shown that the PCET in Ru(II)-TyrOH is dominated by a concerted electron transfer/deprotonation reaction mechanism over the whole pH-range studied. This was deduced from the characteristic, although not fully understood, free energy pH-dependence of the CEP reaction. As the pH increases the CEP driving force increases and the electron transfer rate constant is enhanced in response. This type of behaviour is expected from the Marcus equation and the pH rage investigated actually covers a small part of a Marcus parabola far down in the normal region (figure 5.7). At high pH where the tyrosine part of the molecule is initially deprotonated the electron transfer rate constants are at least two orders of magnitude higher and independent of pH. This observation is in line with the proposed high reorganisation energy for the coupled electron transfer/deprotonation reaction occurring at low pH. At pH
= pKa(TyrOH) the driving force for CEP and ET’ equals. To account for the two orders of magnitude higher rate constant for ET’ the electronic coupling energy has to be substantially higher or the reorganisation energy be lower in the ET’ reaction compared to the CEP (eq. 4.6). However, as shown below, the rate enhancement is in fact due to a reduced reorganisation energy.

5.5 Mechanistic Control of Proton Coupled Electron Transfer

At pH 10 the driving force for CEP and for ET’ are equal but the ET’ rate constant is 100 times larger than the CEP rate constant, the only difference being the coupling of the electron motion with deprotonation in CEP. In 5.2 I argued that the CEP reaction intrinsically has a higher reorganisation energy than a pure electron transfer reaction in the same system accounting for this difference in rate. By temperature dependent measurements of the electron transfer rate constant the validity of this statement can be verified experimentally.

Figure 5.8: Temperature dependence of the logarithm of the intra molecular electron transfer rate constant for CEP from tyrosine to [Ru(bpy)₃]³⁺ in Ru(II)-TyrOH (open symbols) at pH 6.3 (triangles), 7.0 (squares) and 8.2 (circles) and for ET’ from tyrosinate to [Ru(bpy)₃]³⁺ at pH 12.5 (solid circles) in Ru(II)-TyrOH. The left and right axis is relevant for CEP (at low pH) and ET’ (at high pH) respectively.

Figure 5.8 shows the temperature dependence of the electron transfer rate constant in the temperature range between 10°C and 50°C at pH 8 and at pH 12. Clearly the slope, that corresponds to the activation parameters, is markedly larger at pH 8 then at pH 12. A fit of the temperature dependence of the rate constant to eq 4.6 yields the reorganisation energy if the driving forces are known (chapter 5.2). Treating the driving force as a temperature independent parameter, neglecting entropy contributions to ΔG°, the reorganisation energy for the CEP reaction is 2.0 eV, at pH < pKa(TyrOH), while the reorganisation energy for ET’ at pH 12 is 0.9 eV in line with the
statement above. Although the approximation that entropy contributions are small is commonly adapted in electron transfer reactions this approximation is clearly erroneous in the CEP case since the pH-dependent part of the driving force, that contributes to $-0.059\text{pH eV}$ to the reaction free energy, is all entropy contribution. In paper V we estimated the entropy change for the CEP reaction to approximately zero at pH 0, based on literature data, and thus that the entropy for the CEP reaction corresponds to the mixing entropy of the released proton at the relevant pH. Taking this temperature dependent part of $\Delta G^0$ into account reduces the effective reorganisation energy for the CEP to 1.5 eV. This is still significantly higher than the ET' reorganisation energy. This reorganisation energy was used as a fixed parameter in the fit of the pH-dependence of the electron transfer rate constant to eq 4.6 in figure 5.5 and 5.7. The electronic coupling energy, extracted from the temperature dependence data, on the other hand is not significantly affected by the coupling of the proton motion to the electron transfer reaction, i.e. the coupling energy for CEP and ET' is approximately the same.

Figure 5.9: Marcus parabolas for CEP (right parabola) and the ET (left parabola) for the PCET reaction from tyrosine to [Ru(bpy)$_3$]$_3^{3+}$ in Ru(II)-TyrOH. The CEP parabola is shifted relative to the ET parabola due to a higher reorganisation energy for CEP, $\lambda_{\text{CEP}} = 1.5$ eV and $\lambda_{\text{ET}} = 0.9$ eV, making CEP slower than ET for the same driving force in the Marcus normal region. However, the CEP driving force is higher than the driving force for ET by an amount of $-\Delta(\Delta G^0)$ indicated by the arrow for the situation at pH 7. $-\Delta G^0$ for ET is $-0.2$ eV and with the increased driving force for CEP, ET is not competitive unless $-\Delta(\Delta G^0)$ is reduced to 0.25 eV corresponding to a pH of 4. Increasing the driving force for both reactions makes the ET reaction competitive (dotted arrow) due to the parabolic dependence of $\ln(k)$ with $-\Delta G^0$, i.e. the rate constant for a low reorganisation energy reaction increases more than for a high reorganisation reaction for the same increase in driving force.

For the primary ET-step in the ETPT mechanism the reorganisation energy is expected to be approximately the same as for ET'. This has also been verified both experimentally (paper III, see section 5.5.2) and by theoretical calculations$^{85}$. Despite the higher reorganisation energy the CEP is favoured over ET due to the higher driving-force for CEP. Figure 5.9
illustrates the kinetic situation in Ru-TyrOH. The left and right Marcus parabolas correspond to the ET (or ET’) reaction and the CEP reaction respectively. The CEP parabola is shifted relative to the ET parabola due to the higher reorganisation energy for the CEP reaction. For the same driving force CEP is never competitive in the Marcus normal region. However, at pH > pKa(TyrOH\textsuperscript{+}) the driving force for CEP is larger than for ET by an amount given by \(-\Delta(G^0)\), eq 5.14, indicated by the arrows in the figure. (The length of the arrow is for the situation at pH 7).

\[
- \Delta(G^0) = -(\Delta G^0_{\text{CEP}} - \Delta G^0_{\text{ET}}) = \frac{RT \ln 10}{F} (pH - pKa(\text{TyrOH}^{\text{+}}))
\]

(5.14)

Clearly the CEP reaction rate constant is higher than the ET rate constant. For the ET to be competitive a \(-\Delta(G^0)\) less than 0.25 eV is necessary corresponding to the situation at pH 4. We thus expect ET and CEP to be equally fast at pH 4. Another way of reducing \(-\Delta(G^0)\) would be to increase the pKa of the oxidised electron donor. This was done in paper III by replacing tyrosine with tryptophan as electron donor (see next section). Figure 5.9 also suggests a third approach to favour the ETPT reaction path. If the free energy of reaction is decreased for both CEP and ET a larger \(-\Delta(G^0)\) is required for the high-reorganisation energy CEP reaction to compete with the less restrained ET reaction (dotted arrow figure 5.9). Thus an increased driving force for both reaction paths favours the electron transfer first reaction. Such an over-all change in driving force was accomplished by altering the Ru\textsuperscript{2+}/Ru\textsuperscript{3+}-potential of the electron acceptor unit (Paper III)(see section 5.5.2).

5.5.1 Mechanistic switch by altered electron donor properties

Tryptophan has its pKa-values shifted to higher values relative to tyrosine, both for the reduced and the oxidised form. The pH-dependent reduction potential above the pKa for TrpH\textsuperscript{+} is about the same as for tyrosine. The difference in formal potential for the proton coupled redox process for the two amino acids is only about 90 meV with tyrosine being slightly easier to oxidise). Using the same electron acceptor, [Ru(bpy)\textsubscript{3}]\textsuperscript{3+}, the energetics for the CEP reaction is therefore approximately the same. In contrast, due to the higher pKa for TrpH\textsuperscript{+}, the driving force for pure ET is greatly enhanced by the change from tyrosine to tryptophan as electron donor, from \(-0.2\) eV for tyrosine to 0.11 eV for tryptophan. Thus by changing from tyrosine to tryptophan the ETPT path is selectively favoured energetically compared to the CEP path.
Figure 5.10 shows the pH-dependence of the electron transfer rate constant for the intramolecular electron transfer from tryptophan to [Ru(bpy)₃]³⁺ in Ru(II)-TrpH (figure 5.10). Below the pKa for TrpH⁺ the rate constant is independent of pH as expected since no protons are involved in the reaction, i.e. the oxidised tryptophan does not dissociate. Using the [Ru(NH₃)₆]³⁺ electron acceptor the spectrum of a protonated tryptophan radical is seen as the electron transfer product (figure 5.11 upper spectrum).

Figure 5.10: Structure of Ru(II)(bpy)₂(4-Me-4’-(CONH-L-tryptophan ethyl ester)-2,2’-bpy) (Ru(II)-TrpH) (left). pH-dependence of the intra-molecular electron transfer rate constant for electron transfer from tryptophan to [Ru(bpy)₃]³⁺ in Ru(II)-TrpH (left). At low pH, below the pKa for the oxidised Tryptophan (pKa(TrpH⁺) = 4.7), electron transfer is not coupled to deprotonation and the rate constant is independent of pH. Also above this pH a pH-independent rate constant is found up to pH about 9 indicating a ETPT’ reaction path. At still higher pH the CEP reaction path starts to compete with ETPT giving a pH dependent rate constant.

Figure 5.11: Transient absorption spectra after laser excitation and oxidative quenching by [Ru(NH₃)₆]³⁺ of the [Ru(bpy)₃]³⁺ part of Ru(II)-TrpH showing the spectra of the primary and secondary products formed in the electron transfer from TrpH to [Ru(bpy)₃]³⁺ at pH 3 (upper spectrum), 8 (centre spectrum) and 12 (lower spectrum). In the upper transient spectrum the spectrum matches that of a protonated tryptophan radical. This radical is both primary and final product since at this pH TrpH⁺ does not deprotonate. In the centre spectrum the protonated radical is formed transiently (solid line) but decays with a rate constant of c.a. 80 ns to the spectrum of a deprotonated tryptophan radical (line with circles). Finally at pH 12 the deprotonated radical is formed as primary product.
As the pH is increased above the pKa the reduction potential for tryptophan decreases due to the coupling to deprotonation and thus the driving force for the overall proton coupled electron transfer reaction increases. The electron transfer rate constant however remains constant up to pH about nine, contrary to the behaviour in Ru(II)-TyrOH where the electron transfer rate constant reflects the pH-dependence of the formal potential at all pH investigated. As discussed previously either an ETPT mechanism or a PTET mechanism could explain a pH-independent rate constant. However, for the same reason as in the tyrosine case, the PTET reaction path can be ruled out due to slow deprotonation rates from the reduced tryptophan. An ETPT path therefore has to account for the pH-independent rate constant. This conclusion is further supported by the transient absorption spectrum of the electron transfer products where the protonated radical is formed as an intermediate that converts to the deprotonated form within 80 ns (figure 5.11 centre spectrum).

A further increase in pH, pH > 9, gives a pH dependent electron transfer rate constant that increases with pH in accord with the CEP mechanism. At pH about 11 the pH-dependent part of the rate constant equals the pH-independent rate constant at low pH. Thus, at pH 11 the increased driving force for CEP (eq 5.14) is large enough to balance out the high reorganisation energy and to compete efficiently with the low reorganisation energy ETPT reaction path. At pH 12 where CEP dominates the primary as well as the final product seen in the transient absorption spectrum is the deprotonated tryptophan radical formed as an intermediate at lower pH (figure 5.11 lower spectrum).

By replacing tyrosine with tryptophan the ETPT path is selectively favoured energetically due to the lower dissociation constant for oxidised tryptophan compared to tyrosine. The pH-independent ETPT path dominates the kinetics at most pH and a \(-\Delta(G^0)\) of 0.37 eV is required for the CEP to be as fast as the ETPT mechanism. Thus at pH 11, corresponding to \(-\Delta(G^0) = 0.37\) eV, the higher reorganisation energy for CEP compared to ET is exactly balanced by the increased driving force for CEP.

5.5.2 Mechanistic switch by altered electron acceptor properties

The competition between CEP and ETPT is regulated by the relative effect of the high reorganisation energy for CEP and the low driving force for ET. Intuitively the importance of the increased driving force for CEP is less if the reaction free energy is large for both reactions. This intuitive statement was given a more thorough explanation earlier in this chapter (fig. 5.9). By increasing the reduction potential of the donor both mechanisms gains driving force but the increase in reaction rate is expected to be larger for the ET reaction because \(\frac{d\ln k_{ET}}{d(-\Delta G^0)}\) is larger when \(\lambda\) is smaller (figure 5.9).
By introducing electron withdrawing ester groups on the non-linking bipyridines co-ordinated to Ru$^{2+}$, forming Ru$^{3+}_{ester}$-TyrOH (figure 3.4), the Ru$^{3+}_{ester}$ reduction potential for the ruthenium moiety is increased by 0.25 eV relative to the unsubstituted ruthenium part in Ru(II)-TyrOH. Thus the driving force for ET and CEP is increased by 0.25 eV.

From the pH dependence of the electron transfer rate constant (figure 5.12) and from the discussion in 5.5.1 it is evident that also this modification of the reactant induces a mechanistic switch. At low pH the rate constant is independent of pH characteristic for the ETPT mechanism (figure 5.12 segmented, straight line). This mechanism dominates up to pH about 10 where the pH dependent CEP rate constant (figure 5.12 segmented, curved line) becomes equally fast. At even higher pH the concerted reaction is expected to dominate. However, the pH range is limited by the titration of tyrosine to tyrosinate as in Ru(II)-TyrOH but in Ru$^{3+}_{ester}$-TyrOH electron transfer from the tyrosinate could not be resolved because it was faster than the initial photo-oxidation of the Ru-moiety.

![Figure 5.12: pH-dependence of the electron transfer rate constant for electron transfer from tyrosine to [Ru(bpy)$_3$]$^{3+}$ in Ru$^{3+}_{ester}$-TyrOH. At low pH the rate constant is independent of pH, characteristic for an ETPT' reaction path. At higher pH the CEP reaction path starts to compete with ETPT' giving a pH dependent rate constant. The relative contributions to the over-all rate constant from CEP (curved) and ETPT' (straight) are shown as segmented lines and the solid line is a theoretical function for the pH-dependence. Relative contributions were extracted from the fit of the pH-dependence of the electron transfer rate constant. This pH dependence was reproduced with two rate constants, one pH independent ETPT rate constant and one pH-dependent CEP rate constant. The pH-dependence of the CEP rate constant was accounted for by using a pH-dependent driving force in the Marcus equation (eq. 4.6) according to the procedure outlined in section 5.4. In the fit the reorganisation energy for CEP was fixed to 2.4 eV according to the temperature dependence data (see text). At pH about ten the ETPT' rate and the CEP rate equals as can be seen from the crossing of the ETPT' and the CEP contributions to the rate constant.](image-url)
Temperature dependence data for the PCET' reaction at pH 2.7, where the ETPT' mechanism dominates completely, and at pH 8.8, where both ETPT' and CEP contributes to the PCET rate constant, are shown in figure 5.13. At the higher pH the temperature dependence is stronger than at low pH indicating a higher activation energy for the composite rate constant compared to the pure ETPT' reaction. Since the ETPT' reaction is independent of pH the contribution to the rate constant from the ETPT' mechanism at pH 8.8 could be subtracted using the low pH temperature data giving the temperature dependence of the CEP reaction alone (figure 5.13 open circles). By fitting the temperature dependent data to eq 4.6 the electronic coupling energy and the reorganisation energy for ETPT' and for CEP could be determined (table 5.2). While the electronic coupling energy are approximately the same for the two mechanisms the concerted reaction has a much higher reorganisation energy than the electron transfer first mechanism, $\lambda_{ETPT} = 1.2 \text{ eV}$ and $\lambda_{CEP} = 2.4 \text{ eV}$.

![Figure 5.13: Temperature dependence of the logarithm of the electron transfer rate constant for electron transfer from tyrosine to $[\text{Ru(bpy)}_3]^3^+$ in Ru Ester(II)-TyrOH at pH 2.7 (solid circles) where only ETPT' is significant, and 8.8 (solid squares) where both ETPT' and CEP contributes to the rate constant. Open circles are the difference of the ETPT' rate constants (from pH 2.7 data) from the composite rate constants (from pH 8.8. data). Solid lines are fits to eq. 4.6 while the segmented line is a guide for the eye.](image)

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>$H_{rp}$ (cm$^{-1}$)</th>
<th>$\lambda$ (eV)</th>
<th>$-\Delta G^{0s}$ (eV)</th>
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<td>5</td>
<td>1.2</td>
<td>0.05</td>
</tr>
<tr>
<td>CEP</td>
<td>7</td>
<td>2.4</td>
<td>0.58</td>
</tr>
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Table 5.2: Electronic coupling energies ($H_{rp}$), reorganisation energies ($\lambda$) and driving forces ($-\Delta G^{0s}$) (at pH 7 for CEP) for ETPT' and CEP for the electron transfer from tyrosine to $[\text{Ru(bpy)}_3]^3^+$ in Ru Ester(II)-TyrOH deduced from the temperature dependence of the electron transfer rate constant.
Figure 5.14 depicts the kinetic competition between the two mechanisms with the parameters (electronic couplings and reorganisation energies) determined from the temperature measurements. With a driving force for the ET reaction, limiting the ETPT' rate, of 0.05 eV deduced from the reduction potential for RuEster(II) and tyrosine according to eq 5.4 the rate constant for ETPT' is on the order of $10^5$ in line with the experimentally found rate constant. For the CEP reaction to be equally fast a $-\Delta(\Delta G^0)$ of 0.68 eV corresponding to a pH of 9.5 is required. This switching point is very close to the experimentally found pH = 10 from the pH-dependent data where the mechanisms are equally fast (figure 5.12).

Figure 5.14: Summarising picture of what determines the choice of mechanism in the competition between the sequential electron transfer followed by deprotonation mechanism (ETPT') and the concerted electron transfer/deprotonation mechanism (CEP). Due to the coupling of the electron motion with deprotonation the CEP reaction inherently has a high reorganisation energy. The Marcus parabola for the CEP (black line) is therefore shifted to the right relative to the parabola for the rate limiting ET reaction in ETPT' (gray line). Opposing the effect of the high reorganisation energy the CEP reaction is energetically more favourable than ET by $-\Delta(\Delta G^0)$, indicated by the arrows. In RuEster(II)-TyrOH the ET driving force is 0.05 eV which gives a rate constant of about $10^5$ s$^{-1}$ (starting point for the lower arrow). In order for the CEP reaction to be equally fast $-\Delta(\Delta G^0)$ has to be about 680 meV corresponding to a pH of 9.5 which agrees with the switching point determined from the pH-dependent data (figure 5.12). If a stronger oxidant is used the driving force for ETPT' (starting point for the upper arrow) and CEP increases by the same amount. However as indicated by the grey arrow a larger $-\Delta(\Delta G^0)$ is required for the CEP to compete with ETPT' in this case. A large overall driving force thus favours the low reorganisation energy ETPT' reaction over the CEP while the high reorganisation energy CEP is favoured by a high pH, i.e. a large $-\Delta(\Delta G^0)$. 

Figure 5.14: Summarising picture of what determines the choice of mechanism in the competition between the sequential electron transfer followed by deprotonation mechanism (ETPT') and the concerted electron transfer/deprotonation mechanism (CEP). Due to the coupling of the electron motion with deprotonation the CEP reaction inherently has a high reorganisation energy. The Marcus parabola for the CEP (black line) is therefore shifted to the right relative to the parabola for the rate limiting ET reaction in ETPT' (gray line). Opposing the effect of the high reorganisation energy the CEP reaction is energetically more favourable than ET by $-\Delta(\Delta G^0)$, indicated by the arrows. In RuEster(II)-TyrOH the ET driving force is 0.05 eV which gives a rate constant of about $10^5$ s$^{-1}$ (starting point for the lower arrow). In order for the CEP reaction to be equally fast $-\Delta(\Delta G^0)$ has to be about 680 meV corresponding to a pH of 9.5 which agrees with the switching point determined from the pH-dependent data (figure 5.12). If a stronger oxidant is used the driving force for ETPT' (starting point for the upper arrow) and CEP increases by the same amount. However as indicated by the grey arrow a larger $-\Delta(\Delta G^0)$ is required for the CEP to compete with ETPT' in this case. A large overall driving force thus favours the low reorganisation energy ETPT' reaction over the CEP while the high reorganisation energy CEP is favoured by a high pH, i.e. a large $-\Delta(\Delta G^0)$. 

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In photosystem II tyrosine$_Z$ donates an electron to the oxidised photosensitizer P680$^+$. In order to study the electron transfer from tyrosine without the complicating S-state dependence of tyrosine$_Z$ oxidation, PSII particles depleted of manganese can be used. Manganese depletion can be accomplished by tris-wash-treatment$^{93,94}$, low Cl$^-$ treatment$^{95}$ or by exposing the intact system to high pH$^{96}$. Depletion of manganese blocks re-reduction of tyrosine and of course also oxidation of the oxygen-evolving complex thus preventing S-state progression and water oxidation activity. Mn-depletion also decreases the electron transfer rate from tyrosine$_Z$ to P680$^+$ by several orders of magnitude, from 50-250 ns in the intact system to 1-200 $\mu$s in the inactivated system$^{96}$.

Interestingly the electron transfer rate constant for tyrosine$_Z$ oxidation in Mn-depleted PSII particles is strongly dependent on the pH of the surrounding aqueous solution (figure 6.1). At low pH the electron transfer rate constant increases monotonously with pH, similar to the low-pH behaviour in Ru(II)-TyrOH. This pH dependent phase titrates to a pH-independent, higher, rate constant with an apparent pKa of 7. The two
kinetic phases differ not only in rate constant and dependence on pH but also in activation energy (Ea) and deuterium isotope effect. While the slow phase slows down significantly by deuteration (kH/kD = 2.5) and is highly activated (Ea = 0.30 eV at pH 6.0) the fast phase shows almost no deuterium isotope effect (kH/kD ≤ 1.1) and a much lower activation energy (Ea = 0.15 eV). Qualitatively tyrosineZ oxidation thus shows the same features as the Ru(II)-TyrOH model complex with a pH-dependent, highly activated and isotope dependent fraction dominating at low pH and a pH-independent, less activated and isotope independent fraction dominating in a high pH region. In the intermediate pH-region, the titration region, the two phases coexists.

Due to the similarity in pH-dependence, deuterium isotope effect and activation energy between tyrosineZ oxidation in manganese depleted PSII and in Ru(II)-TyrOH in the low pH-regime it is likely that the PCET mechanism is the same, i.e. the electron transfer and the deprotonation occurs in one single concerted reaction step. However the pH-dependence in the CEP mechanism is due to the release of the phenolic proton directly to bulk water. Interpreting the pH-dependence of tyrosineZ oxidation as being a consequence of the CEP mechanism by necessity suggests that tyrosineZ is in direct contact with bulk water and that the proton is released to the bulk without any intermediate proton carriers. Accordingly the rate of proton release to bulk has to equal the rate for ET from tyrosineZ to P680+. At pH where the pH-dependent fraction dominates the rate of proton release is in fact within a factor of two equal to the electron transfer rate and importantly it shows the same increase in rate with pH (figure 6.1). As the fraction of the non-pH-dependent rate constant increases, the rate of proton release slows down to a constant rate above pH 8 of 2.5×10³ s⁻¹ indicating that the proton release is not simultaneous with the electron transfer reaction in the high pH-region.

Although the proton release is slowed down in the high pH regime one proton per electron transfer reaction is still released to the bulk at pH > 5. In reference 96 this was explained by proton release from peripheral amino acids, not directly involved in the electron transfer reaction, as a consequence of the charge transfer. An electron transfer that does not give rise to a direct transfer of a proton to the bulk would create a positive charge at the electron donor site. This positive charge could in principle induce a pKa-shift of peripheral amino acids at the protein-bulk interface that deprotonates subsequent to the electron transfer reaction. However, the involvement of at least three amino acids was required to account for the proton release and I believe this explanation for the proton release at pH > 7 to be unlikely.

In order to build up a charge at the tyrosine site upon electron transfer to P680+, either tyrosineZ has to be initially deprotonated or the phenolic tyrosine proton has to be retained at, or near, tyrosineZ upon oxidation. The tyrosine radical is very acidic, with a pKa of approximately –2, and with the
above conclusion that tyrosine\textsubscript{Z} is in direct contact with bulk water it is difficult to realize how a protonated radical could be retained. Alternatively an electron transfer from the tyrosinate would give a charge-change at the tyrosine site from –1 to 0. However this suggests that the titration (figure 6.1) with an apparent pKa of 7 is due to direct titration of tyrosine. Such a low pKa-value seems inconsistent with tyrosine. Moreover, if tyrosine is deprotonated we expect the effect of the titration to be the same in PSII as in the Ru(II)-TyrOH reference compound. While the titration at pH 7 in PSII gives a pH-independent rate constant one order of magnitude faster than the pH dependent rate constant the titration in Ru(II)-TyrOH increases the rate constant by two orders of magnitude. Moreover the activation energy in PSII for the pH-independent fraction is 0.15 eV compared to 0.3 eV for the pH-dependent part. The titration in Ru(II)-TyrOH gives a much more pronounced change in activation energy due to tyrosine deprotonation.

Figure 6.2: Mechanistic model for tyrosine\textsubscript{Z} oxidation in Mn-depleted PS II. At all pH values electron transfer from tyrosine\textsubscript{Z} to P680\textsuperscript{+} is concerted with deprotonation (CEP). In hydrogen bond distance to tyrosine\textsubscript{Z} a base (B\textsuperscript{-}), with a pKa of 7 for the corresponding acid (HB), is situated. When protonated the base is incompetent as hydrogen bond acceptor to the phenolic tyrosine proton and the tyrosine proton is released directly to the surrounding solvent upon tyrosine oxidation (upper part). At pH above the base pKa a hydrogen bond is formed between the base and tyrosine and in this case the tyrosine proton is released to the base (lower part).

Instead our comparison of tyrosine\textsubscript{Z} oxidation with the model complex tends to favour another proposed mechanistic model for tyrosine\textsubscript{Z} oxidation\textsuperscript{96,31} where the titration at pH 7 corresponds to the deprotonation of a base in close proximity to tyrosine\textsubscript{Z} (figure 6.2). A likely candidate being a histidine, His190, which is known to be important for rapid electron transfer from tyrosine\textsubscript{Z} in the intact PSII.\textsuperscript{32,33,34} The apparent pKa of 7 is also reasonable for a histidine that in its free form has a pKa of 6 and from a recent PSII crystal structure His 190 is in fact in hydrogen bond distance to tyrosine\textsubscript{Z}\textsuperscript{20}. When titrated to its deprotonated form the base forms a hydrogen bond to the phenolic tyrosine proton (figure 6.2). Upon electron transfer to
P680$^+$ the tyrosine proton is transferred to the base while at low pH, where the base is protonated and blocked as hydrogen bond acceptor, the proton is released to the bulk thus accounting for the pH-dependence. Since the proton is transferred to a base in the high pH region the PCET does not depend on the pH of the bulk, hence the pH-independent PCET rate constant.

The titration at pH 7 suggests that the protonated base (HB) is unstable with respect to deprotonation at pH above 7 when tyrosine$_Z$ is in its neutral protonated form. PCET from tyrosine to P680$^+$ gives, within this model, a neutral tyrosine radical. The difference in coulombic interaction of a protonated neutral tyrosine and a neutral tyrosine radical with the base is expected to be minor since both tyrosine forms are neutral. Therefore also in the PCET product-state the base is expected to be unstable with respect to deprotonation. The one proton released per electron transfer reaction discussed above can then be understood as the transfer of the phenolic tyrosine proton to the bulk via the base. In this case, as opposed to the low pH behaviour, the rate of proton release is not limited by the PCET rate but instead by the deprotonation rate of a base with a pKa of 7. In the titration region (pH $\approx$ 7) two deprotonation rate-constants are expected with pH-dependent amplitudes, one corresponding to direct proton release from tyrosine$_Z$ (pH < 7) and one for proton release from the base (pH > 7). Also with the alternative interpretation for proton release discussed above more than one rate constant is expected. However, in reference 96 only mono-exponential rates are given indicating that different contributions to proton release could not be resolved in this work. The observed pH-dependence of the deprotonation rate constant in the titration region can then be understood as being due to a single exponential fit to biexponential data with pH-dependent amplitudes. Following the discussion in chapter 5.1 the deprotonation rate constant for deprotonation of a base with a pKa of 7 is expected to be on the order of $1 \times 10^4$ s$^{-1}$, assuming diffusion controlled protonation rates. This is close to the proton release rate found experimentally in the high pH region ($2.5 \times 10^3$ s$^{-1}$).

Titration of a nearby base that forms a hydrogen bond to tyrosine$_Z$ when deprotonated thus accounts for the pH dependence of the PCET rate constants as well as the proton release rates. The hydrogen bond also has to account for the rate enhancement, the decreased activation energy for the PCET rate constant as well as the decreased deuterium isotope effect induced by the hydrogen bond.

A hydrogen bond can affect the PCET reaction in several ways. The base may increase rate of tyrosine deprotonation$^{31}$ thus opening up the PTET reaction path. In this mechanism electron transfer occurs only from the fraction of tyrosines that are deprotonated. This fraction is given by $10^{-\Delta pK_a}$, where $\Delta pK_a = pK_a(\text{TyrOH}) - pK_a(\text{BH})$, and the PTET rate constant is given by this fraction times the electron transfer rate constant for the electron transfer from tyrosinate to P680$^+$. Since the proton equilibrium is established
prior to electron transfer this model effectively accounts for the lack of deuterium isotope effect. However with a histidine base the pKa-difference is 4 (Paper II) and thus the electron transfer rate constant has to be in the order of $1 \times 10^{10}$ s$^{-1}$ which is more than two orders of magnitude faster than the electron transfer rate constant for tyrosinate oxidation in Ru(II)-TyrOH. Since the rate constants in the low pH regime are comparable in Mn depleted PSII and in the model complex such a high rate for tyrosinate oxidation in PSII seems unrealistic. Also the activation energy of 0.15 eV in the high pH region is difficult to reconcile with this model, where the activation energy is given by $0.059 \Delta \text{pKa} + \text{Ea}(k_{ET})$ (Paper II). In the model complex $\text{Ea}(k_{ET})$ is 0.05 eV. Assuming that the electron transfer activation energy is the same in Mn depleted PSII $\Delta \text{pKa}$ has to be less than 2 which seems to small for the tyrosine-histidine couple.

Hydrogen bonding of the tyrosine proton also affects the concerted reaction. Since the proton is no longer released directly to the solvent the driving force does not depend on the bulk properties of water but instead on the pKa value of the base. However, since the base is initially deprotonated, deprotonation of the protonated base is energetically favoured. That is, the free energy is smaller if the proton is in the solvent than on the base and it is consequently energetically more favourable to release the proton to the solvent than to the base. Hydrogen bonding therefore always decreases the driving force for the CEP reaction compared to direct proton release to bulk. The rate enhancement upon hydrogen bond formation can thus not be accounted for by reaction free energy arguments.

As discussed in section 5.3 the concerted electron transfer/deprotonation reaction with proton release to bulk has an intrinsically high reorganisation energy due to the proton motion. If the proton is transferred to a base, rather than to the solvent, the proton distortion distance is probably reduced and also solvent reorganisations due to the proton transfer is less since the solvent no longer has to accommodate the released charge. Hydrogen bonding must therefore reduce the CEP reorganisation energy. This effect could in principle account for the decreased activation energy and the increased rate upon hydrogen bond formation. In order to test this hypothesis well defined model compounds, which includes hydrogen bond acceptors to the phenolic proton, have to be studied in detail. Investigation of hydrogen bonded model complexes would also reveal the effect of deuterium isotope exchange on PCET reactions in hydrogen bonded systems.
7 Effect of Hydrogen Bonds in PCET and The Rate Ladder

From the discussion in the previous chapter it is clear that biomimetic systems modelling PCET reactions in biological systems has to be able to reproduce the effect of hydrogen bonds to the transferring proton. In 2-hydroxy-phenyl-acetic acid (I) and Salicylic acid (II) (figure 7.1) strong intra-molecular hydrogen bonds between the carboxylic group and the phenolic proton are formed. To study the effect of hydrogen bonds on phenol oxidation these model compounds have been used, together with their para-substituted reference compounds (I’ and II’ respectively), as electron donors to the flash-quench oxidised [Ru(bpy)3]2+-sensitizer (Paper IV).

![Figure 7.1: Structures of 2-hydroxy-phenyl-acetic acid (I) and Salicylic acid (II) together with their para-substituted reference compounds, I’ and II’.](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>I</th>
<th>I’</th>
<th>II</th>
<th>II’</th>
</tr>
</thead>
<tbody>
<tr>
<td>pKa(PhenOH)</td>
<td>10.9*1</td>
<td>10.3*2</td>
<td>13.5*2</td>
<td>9.3*2</td>
</tr>
<tr>
<td>pKa(COOH)</td>
<td>4.3*2</td>
<td>4.3*2</td>
<td>3.1*2</td>
<td>4.5*2</td>
</tr>
<tr>
<td>(E_0(\text{PhenO}^+/\text{PhenO}))</td>
<td>0.71</td>
<td>0.75</td>
<td>0.77</td>
<td>0.9</td>
</tr>
<tr>
<td>(k_b) (M^{-1}s^{-1}))</td>
<td>3.6\times10^9</td>
<td>3.2\times10^9</td>
<td>3.0\times10^9</td>
<td>3.0\times10^9</td>
</tr>
<tr>
<td>(k_a) (M^{-1}s^{-1}))</td>
<td>-</td>
<td>4.8\times10^5</td>
<td>-</td>
<td>1.7\times10^5</td>
</tr>
<tr>
<td>(k_{HB}) (M^{-1}s^{-1}))</td>
<td>4.8\times10^7</td>
<td>-</td>
<td>9.3\times10^6</td>
<td>-</td>
</tr>
<tr>
<td>(\beta)</td>
<td>0.3*5</td>
<td>0.3</td>
<td>0.3*5</td>
<td>0.3</td>
</tr>
</tbody>
</table>

*1 data from ref. 99, *2 data from ref. 100, *3 Determined by puls radiolysis with 4-I-phenolate as reference (see paper IV), *4 extracted from kinetic data (see text), *5 Locked to the value for the respective reference compound.
Figure 7.2 shows the pH dependence of phenol oxidation ($k_{ox}$) by bimolecular electron transfer from the phenols to $[\text{Ru(bpy)}_3]^3+$ extracted from the pseudo first order electron transfer rate constant ($k_{PFO} = k_{ox}[\text{PhenOH}]$) determined with the flash-quench method described in 3.4. The titration of the phenols to phenolates centred at the respective pKa-values for the different phenols pKa(PhenOH) (table 7.1) dominates the pH-dependence of the electron transfer rate constant. Contrary to the previous measurements on covalently linked $[\text{Ru(bpy)}_3]^{2+}$-tyrosine complexes reported in this thesis the individual rate constants for electron transfer from protonated and the deprotonated phenol-form can not be studied separately since the electron donor and the electron acceptors in this case are not covalently linked.

![Figure 7.2: pH-dependence of the observed rate constant for bimolecular electron transfer from I (A), I' (B), II (C) and II' (D) to photo oxidised $[\text{Ru(bpy)}_3]$^{2+}. Dotted lines are fits to eq. 7.1 with $k_a$, $k_a'$ and $k_b$ independent of pH while the solid line is a the same fit but with a pH-dependent $k_a$ and $k_a'$ according to eq. 7.4 accounting for a pH-dependence of the CEP rate constant for phenol oxidation with proton release to bulk water. The pH-independence of phenol oxidation (pKa(COOH) < pH < pKa(PhenOH)) in I and II is attributed to a hydrogen bond between the phenol and the deprotonated carboxylate group. At pH > pKa(COOH) the hydrogen bond is broken and the rate constant for phenol oxidation is again pH-dependent.

Instead the observed electron transfer rate constant ($k_{ox}$) is given by the sum of rate constants for phenol- and phenolate oxidation weighted by their pH-dependent fractions (eq 7.1). Additionally titration of the carboxylic group is taken into account by allowing two rate constants for phenol oxidation with pH-dependent fractions, for phenols with protonated and deprotonated carboxylate groups.
\[ k_{\text{tot}} = f_a k_a + f_b k_b + f_{a'} k_{a'} \]  \hspace{1cm} (7.1)

Here \( k_a \) and \( k_b \) correspond to the rate constant for phenol- and phenolate oxidation respectively and \( k_{a'} \) corresponds to the rate constant for phenol oxidation with protonated carboxylate groups. The phenol fractions, \( f_a \) and \( f_{a'} \), and phenolate fractions, \( f_b \), are given by \( \text{pKa(PhenOH)}, \text{pKa(COOH)} \) and the \( \text{pH} \) according to eq 7.2a, 7.2b and 7.2c.

\[ f_{a'} = \frac{1}{1 + 10^{\text{pH}-\text{pKa(COOH)}}} \]  \hspace{1cm} (7.2a)

\[ f_a = \frac{1}{1 + 10^{	ext{pKa(COOH)}-\text{pH}} + 10^{\text{pH}-\text{pKa(PhenOH)}}} \]  \hspace{1cm} (7.2b)

\[ f_b = \frac{1}{1 + 10^{\text{pKa(PhenOH)}-\text{pH}}} \]  \hspace{1cm} (7.2c)

A titration of three pH-independent rate constants according to eq 7.1 where the increase in oxidation rate with \( \text{pH} \) is due to the pH-dependent fractions\(^{101,102} \) can however not account for the observed pH-dependence (figure 7.2 segmented line). The deviation from this simple model is particularly apparent in the reference compounds at \( \text{pH} \) below the phenol pKa.

From the work with covalently linked \([\text{Ru(bpy)}_3]^{2+}\)-Tyrosine complexes we know that the rate constant for a concerted electron transfer/deprotonation reaction with direct proton release to bulk water also shows a pH dependence. In the reference compounds, I' and II', we therefore expect the rate constant for phenol oxidation \( (k_a) \) to depend on \( \text{pH} \) according to equation 4.6 with a pH-dependent driving force given by the \([\text{Ru(bpy)}_3]^{3+}/[\text{Ru(bpy)}_3]^{2+}\) reduction potential and the phenol formal potential, i.e. eq. 5.11. A mechanism other than CEP is implausible since the phenol pKa is similar to that of tyrosine excluding PTET as above and the reduction potential is slightly higher for the phenols (table 7.1) compared to tyrosine making an ETPT mechanism unlikely. (The ETPT mechanism is favoured by a higher driving force and thus by a lowed phenol potential (see section 5.5)). With the complicating titration of the phenol however a fit of the pH-dependence, eq 7.1, with a pH-dependence of \( k_a \) and \( k_{a'} \) according to equation 4.6 is too difficult and a simplified expression for the pH-dependence of \( k_a \) has to be used.

The pH-dependence of the CEP reaction with proton release to bulk water is due to a pH-dependent driving force and the available pH-range covers a small part of a Marcus parabola far down in the normal region (figure 5.7). In such a small driving force range a Taylor expansion around \( \Delta G^0' \) of the Marcus equation with respect to \( \Delta G^0' \) is a good approximation and gives equation 7.3.
\[
\ln k_{ET} = \ln \frac{2\pi H_{\text{sp}}}{\hbar} \frac{2}{\sqrt{4\pi k_B T \lambda}} - \left( \frac{\Delta G^0_g + \lambda}{4k_B T \lambda} \right)^2 + \left( \frac{\Delta G^0_g + \lambda}{2k_B T \lambda} \right) \left( \frac{\Delta G^0_g - \Delta G^0_b}{2k_B T \lambda} \right)
\]

(7.3)

Thus the logarithm of the electron transfer rate constant increases linearly with \(-\Delta G^0_g\) and since \(d\Delta G^0_g/dpH = -0.059\) meV/pH (eq. 5.11) we expect a linear dependence of \(\ln k_{ET}\) on pH with a slope of \(0.059(\Delta G^0_g + \lambda)/(2k_B T \lambda)\) for the CEP reaction with proton release to bulk. To account for the pH dependence of phenol oxidation \(k_a\) and \(k_a'\) was allowed to vary with pH according to eq 7.4 in agreement with the Taylor expansion result, where \(k_a^0\) is the rate constant for phenol oxidation at pH 0.

\[
k_a = k_a^0 10^{\beta pH}
\]

(7.4)

With the inclusion of two pH-dependent rate constants for phenol oxidation the pH-dependence of the observed rate constant could be reproduced for the reference compounds (figure 7.2 solid line). (In the fit the rate constants \(k_a\), \(k_a^0\) and \(k_a'\) as well as \(\beta\) was allowed to vary while the fractions was given by the fixed pKa-value for the respective phenols). The results of the fit are given in table 7.1. From the pH-dependence it is thus clear that the electron transfer reaction and deprotonation of the phenol occurs concertedly (CEP) in these non-hydrogen bonded phenols. For comparison the corresponding \(\beta\)-value for tyrosine oxidation in Ru(II)-TyrOH for the data in figure 5.5 was very similar, \(\beta = 0.36\).

For the hydrogen bonded phenols, I and III, the pH-dependence is much weaker in the pH region where the phenols are protonated and the carboxylate groups are deprotonated, i.e. at pH where the carboxylate group can act as an internal base to the phenolic proton. At low pH-values below the carboxylate pKa on the other hand the, pH-dependence in I is analogous to that of the reference indicating that the internal hydrogen bond is broken and the proton is released directly to the bulk in a CEP reaction. Irrespective of mechanism the rate constant for phenol oxidation is expected to be independent of pH if the phenolic proton is hydrogen bonded since the transferring proton is no longer transferred directly to bulk water. The weak pH-dependence in the intermediate pH-region could be explained by a small fraction of non-hydrogen bonded phenols. The solid line in figure 7.2 is a fit to eq. 7.1 with \(k_a\) and \(k_a'\) given by eq 7.5 accounting for one pH-dependent fraction from non-hydrogen bonded phenols (\(\alpha\)) and one fraction with an intact intramolecular hydrogen bond (1-\(\alpha\)).

\[
k_a = (1-\alpha)k_{HB} + \alpha k_a^0 10^{\beta pH}
\]

(7.5)
By comparing the rate constants in the reference compounds with the rate constants for the non-hydrogen bonded fraction $\alpha$ could be estimated to 0.2 in I and 0.1 in II.

As stated above all three possible PCET mechanisms, PTET, ETPT and CEP, would give pH-independent rate constants for phenol oxidation in a hydrogen bonded system. However the PTET reaction path can be excluded for kinetic reasons. The over-all rate constant in PTET is given by the phenolate fraction times the rate constant for phenolate oxidation ($k_b$) (eq 7.6), where the fraction is given by the pKa-difference between the phenol and the carboxylate base, $\Delta pK_a = pK_a(\text{PhenOH}) - pK_a(\text{COOH})$ (Table 7.1).

$$k_{PTET} = k_b 10^{-\Delta pK_a} \quad (7.6)$$

This fraction is only $10^{-7}$ and $10^{-11}$ for I and II respectively. To account for the observed rate constant $k_b$ thus has to be $10^{14}$ s$^{-1}$ in I and $10^{18}$ s$^{-1}$ in II which is at least four orders of magnitude larger then the experimentally determined rate constant for phenolate oxidation (table 7.1).

![Figure 7.3: Limiting rate constants for ETPT' in I' and II' and observed rate constants for hydrogen bonded systems, I and II, with $[\text{Ru(bpy)}_3]^3+$ as oxidant (solid circles) and observed rate constants with Br$_2^-$ as oxidant (open circles) for the phenols as a function of PhenO$^x$/PhenO$^-$ potential. The linear correlation of the observed rate constant with $E^0(\text{PhenO}^x/\text{PhenO}^-)$ with the Br$_2^-$ oxidant is indicative of a ETPT' mechanism. The lack of correlation when $[\text{Ru(bpy)}_3]^3+$ is oxidant suggests a different mechanism for phenol oxidation in this case, i.e. CEP.

The ETPT' rate constant correlates with the PhenOH$^{x+}$/PhenOH reduction potential according to eq. 5.4. However this reduction potential is unknown and has to be estimated. It is reasonable to assume that the difference in potential for the protonated and deprotonated redox couple is similar for the different phenols and thus that the ETPT' rate constant also correlates with the PhenO$^x$/PhenO$^-$ potential. Figure 7.3 shows the rate constant $k_{\text{lim}}$ for I and II, as well as the limiting ETPT' rate constants in the reference compounds at the lowest pH values examined, as a function of
PhenO'/PhenO potential. Clearly there is no correlation of the rate constant with \(E^0(\text{PhenO}'/\text{PhenO})\). When a stronger oxidant than \([\text{Ru(bpy)}_3]^{3+}\) was used, \(\text{Br}_2\) (\(E^0(\text{Br}_2/2\text{Br}^-) = 1.7 \text{ V}[101,103]\)), favouring the ETPT' reaction path a clear correlation of the electron transfer rate constant and \(E^0(\text{PhenO}'/\text{PhenO})\) was found (figure 7.3) indicating that the hydrogen bond has no significant effect on the ETPT' rate constant. With the stronger oxidant the rate constant for phenol oxidation in the pH-range 5.5-6.6 was also independent of pH in line with an ETPT mechanism. The difference in behaviour with the \([\text{Ru(bpy)}_3]^{3+}\) oxidant in I and II and their reference compounds can therefore not be explained with an ETPT' reaction thus excluding also this step-wise mechanism. Instead, we propose that the reaction follows a CEP mechanism also in the hydrogen bonded I and II.

In a concerted electron transfer/deprotonation reaction in hydrogen bonded systems the proton is initially transferred to the hydrogen bond acceptor. The CEP driving force, and consequently the rate constant, therefore depends on the difference in pKa of the phenol radical and the carboxylate base in I and II (eq 7.7) and not on pH as in I' and II' (eq 7.8).

\[
\Delta G^0(\text{CEP, HB}) = -(E^0_{\text{Ru}^+/\text{Ru}^{2+}} - E^0_{\text{PhenOH}'/\text{PhenOH}}) + 0.059(p\text{Ka(COOH)-pKa(PhenOH')})
\]

\[
0.059(p\text{Ka(COOH)-pKa(PhenOH)})
\]

\[
0.059(p\text{Ka(COOH)-pKa(PhenOH)})
\]

\[
(7.7)
\]

\[
\Delta G^0(\text{CEP}) = -(E^0_{\text{Ru}^+/\text{Ru}^{2+}} - E^0_{\text{PhenOH}'/\text{PhenOH}}) + 0.059(p\text{H-pKa(PhenOH')})
\]

\[
0.059(p\text{H-pKa(PhenOH)})
\]

\[
(7.8)
\]

Clearly at pH above the carboxylate pKa, in the pH-region where the carboxylate group forms a hydrogen bond to the phenol, proton release to bulk water is energetically more favourable compared to proton release to the base. (In II the CEP reaction is actually slightly endergonic when the proton is released to the carboxylate base). In spite of the lower driving force in the hydrogen bonded phenols, I and II, the rate constant for phenol oxidation in these compounds is equal to (II) or higher than (I) the rate constant in the reference compounds. Because the electronic coupling is independent of both protonation state of the phenol as well as the coupling of the electronic motion to the proton motion, as we shown for the Ru(II)-TyrOH model complexes, it seems unlikely that a hydrogen bond would affect the electronic coupling significantly. The rate enhancement in I and II compared to I' and II' therefore has to be attributed to a reduced reorganisation energy due to the hydrogen bond.
To estimate the effect of hydrogen bonds on the reorganisation energy we can assume that the reorganisation energy for phenol oxidation with proton release to bulk water is the same as for tyrosine oxidation in Ru(II)-TyrOH, 1.5 eV for the same mechanism. This is reasonable since the electron acceptors as well as the electron donor in the two cases are structurally very similar and the reactant distance is probably not very different. In order to account for the relatively high rate constants in the hydrogen bonded systems the reorganisation energy in I and II has to be 1.2 eV and 0.9 eV respectively. An analogous treatment of tyrosine oxidation discussed in chapter 6 yields a reorganisation energy for the proposed hydrogen bonded CEP reaction of 1.1 eV. A hydrogen bond thus seems to significantly reduce the reorganisation energy for the concerted PCET reaction. The CEP mechanism with proton motion through a hydrogen bond thus provides an energy conservative, low energy barrier path that can operate efficiently even for slightly endergonic reactions.

Interestingly a hydrogen bond to the transferring proton not only accounts for the rate enhancement upon titration of the hydrogen-bonded base but also for the reduced deuterium isotope effect in Mn-depleted PS II above the base pKa. In I' and II' the rate constant decreased by a factor of 2.2 and 2.0 respectively by deuteration. This deuterium isotope effect is comparable to the one found in Ru(II)-TyrOH, $k_{H}/k_{D} = 2-2.5$, as well as in the low pH-region for tyrosine oxidation, $k_{H}/k_{D} = 2.5$, in Mn-depleted PS II. For the hydrogen bonded phenols the effect was much smaller, $k_{H}/k_{D} = 1.6$ and $k_{H}/k_{D} = 1.2$ for I and II respectively, and seems to correlate with the hydrogen bond strength. (Calculations show that the hydrogen bond strength is smaller in I than in II).\(^9\) Moreover the deuterium isotope effect in the different phenols seems to correlate with the reorganisation energy (figure 7.4).

![Figure 7.4: Correlation between deuterium isotope effect and estimated reorganisation energies for phenol oxidation in hydrogen bonded systems, I and II and non-hydrogen bonded system I' and II'. The deuterium isotope effect for the non-hydrogen bonded systems is taken as the mean of $k_{H}/k_{D}$ for I' and II'. The solid line is a guide for the eye.](image)
We have thus shown that a concerted electron transfer/deprotonation reaction can account for the pH-dependence, temperature dependence as well as the deuterium isotope effect of tyrosine$Z$ oxidation in Mn-depleted PS II. At low pH the phenolic tyrosine proton is released directly to bulk water giving a rate constant that increases with pH, with a high reorganisation energy and a large deuterium isotope. At higher pH a base in the vicinity of tyrosine$Z$ is deprotonated and forms a hydrogen bond to the phenolic tyrosine proton. This hydrogen bond reduces the reorganisation energy for the CEP as well as the deuterium isotope effect and, since the proton is not released directly to the bulk, the rate constant is independent of pH.

Figure 7.5: Schematic of the Rate Ladder for Proton Coupled Electron Transfer Reactions. The rate constant for CEP (black lines) from phenolic compounds with an intra-molecular hydrogen bond acceptor as a function of pH. At low pH, where the hydrogen bond acceptor is protonated and inefficient as base, the rate constant increases with pH since the phenolic proton is released directly to bulk water. Characteristic of this pH-region is a large reorganisation energy ($\lambda$) (red lines) and a large deuterium isotope effect ($k_H/k_D$) (blue lines) due to the coupling of the electronic motion with deprotonation. In the intermediate pH-region the base is deprotonated and forms a hydrogen bond to the phenolic proton. This hydrogen bond significantly reduces $\lambda$, as well as $k_H/k_D$ for the CEP reaction. Finally when the phenol is deprotonated in the high pH-regime electron transfer is no longer coupled to deprotonation and the rate constant is much higher due to an even lower $\lambda$ in this region.

Figure 7.5 summarises the pH-dependence of phenol oxidation and the characteristics of the different pH-regions in the ‘Rate Ladder’ for Proton Coupled Electron Transfer Reactions. In the low pH regime the phenolic proton is released to bulk water upon oxidation in a CEP mechanism. This reaction has a high intrinsic reorganisation energy and also a large deuterium isotope effect due to the coupling of the electronic motion with
deprotonation. If a base is present that forms a hydrogen bond to the phenolic proton, titration of this base results in an increased rate constant for phenol oxidation. The rate enhancement induced by the hydrogen bond is most likely an effect of a reduced reorganisation associated with the proton motion. As a consequence of the lower proton reorganisation the effect of isotope exchange is also reduced. Finally, when the phenol is deprotonated electron transfer from the phenolate is no longer coupled to deprotonation and the proton distortion reorganisation is absent. Hence the reorganisation energy is even smaller and also the driving force for the reaction is higher resulting in a larger rate constant.
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