Coupled electron transfers in artificial photosynthesis

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Light-induced charge separation in molecular assemblies has been widely investigated in the context of artificial photosynthesis. Important progress has been made in the fundamental understanding of electron and energy transfer and in stabilizing charge separation by multi-step electron transfer. In the Swedish Consortium for Artificial Photosynthesis, we build on principles from the natural enzyme photosystem II and Fe-hydrogenases. An important theme in this biomimetic effort is that of coupled electron-transfer reactions, which have so far received only little attention. (i) Each absorbed photon leads to charge separation on a single-electron level only, while catalytic water splitting and hydrogen production are multi-electron processes; thus there is the need for controlling accumulative electron transfer on molecular components. (ii) Water splitting and proton reduction at the potential catalysts necessarily require the management of proton release and/or uptake. Far from being just a stoichiometric requirement, this controls the electron transfer processes by proton-coupled electron transfer (PCET). (iii) Redox-active links between the photosensitizers and the catalysts are required to rectify the accumulative electron-transfer reactions, and will often be the starting points of PCET.

Keywords: artificial photosynthesis; proton-coupled electron transfer; photosystem II; manganese; tyrosine

1. INTRODUCTION

Photosystem II (PSII) is a key component of the most successful solar energy-converting process on our planet: oxygenic photosynthesis. It uses light energy to accomplish the reduction of plastoquinone and the oxidation of water (Barber 2002; Diner & Rappaport 2002; Goussias *et al.* 2002):

Electronic excitation of the primary chlorophyll donor P₆₈₀ by energy transfer from antenna pigments initiates a chain of electron transfer reactions, which separates oxidative and reductive equivalents over PSII. The four-electron water-oxidation reaction is catalysed by a Mn₄Ca cluster (Ferreira *et al.* 2004; Haumann *et al.* 2005; Loll *et al.* 2005; Yano *et al.* 2006) and gives the organism an abundant source of electrons for the reduction of carbon dioxide to carbohydrates. The principles and many details of the light-harvesting and charge-separation processes are known. In contrast, the structure of the cluster, and the mechanism by which it catalyses water oxidation, are controversial and not yet fully understood.

The reactions and mechanistic principles of PSII provide important inspiration and information for biomimetic chemistry, which attempts to mimic the fundamental photosynthetic processes in synthetic molecular systems (Wasielewski 1992; Ruttinger & Dismukes 1997; Gust *et al.* 2001; Sun *et al.* 2001;

Hammarström 2003; Mukhopadhyay *et al.* 2004; Alstrum-Acevedo *et al.* 2005). The goals are to improve and generalize our understanding and to ultimately pave the way for a molecular solar-energy-conversion technology by artificial photosynthesis. Light-induced charge separation on a single-electron level has been demonstrated in numerous synthetic systems, including multi-unit ‘triads’, ‘tetrads’, etc. (Wasielewski 1992; Gust *et al.* 2001; Baranoff *et al.* 2004; Alstrum-Acevedo *et al.* 2005). Each absorbed photon leads to the transfer of only one electron, however, while full reduction of the ultimate PSII acceptor, Q_B, requires two electrons before it exits to the plastoquinone pool. Water oxidation at the Mn₄Ca cluster is even more demanding as four electrons have to be removed to complete the catalytic cycle. Thus, several sequences of light-induced charge separation on the single-electron level have to be coupled to achieve accumulation of charge, or rather redox equivalents, on a single molecular unit or metal cluster. This we denote *accumulative electron transfer*, to distinguish it from both *multi-step electron transfer* that is a sequence of electron transfer steps on the one-photon/one-electron level and *multiple electron transfer* that is the transfer of two or more electrons in a single reaction step.

Accumulation of oxidizing equivalents on the Mn₄Ca cluster is coupled to the release of protons (Junge *et al.* 2002; Dau & Haumann 2006). Therefore, the increase in oxidation state does not lead to an increase in charge, except probably at the transition from state S₁ to S₂ (figure 1). Thanks to this charge-compensation mechanism, the redox potentials of the different oxidation steps are rather similar. This allows the Tyz_Z radical, which is the intermediate electron donor between P₆₈₀ and the Mn₄Ca cluster, to oxidize

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Figure 1. (a) Schematic of the protein-coupled electron transfer (PCET) reactions on the electron donor side of PSII (based on the structure of Ferreira *et al.* (2004)). In the PCET reaction (1.1), Tyr_Z is oxidized by electron transfer to P₆₈₀⁺ and proton transfer to His190. In the subsequent PCET reaction, Tyr_Z is reduced again by electron transferred from the Mn₄Ca cluster, while it is not clear from where the proton is transferred. (b) The different oxidation states of the Mn₄Ca cluster in the so-called S-state cycle. Each photon excitation leads to a one-step oxidation, and the currently most favoured proton release pattern is indicated.

the cluster in every step of the cycle without wasting an unnecessary amount of free energy in the early steps.

The coupling of electron and proton transfer on the donor side of PSII begins with the oxidation of the tyrosine Tyr_Z, which is the electron donor that regenerates the P₆₈₀ chlorophylls after oxidation. The phenolic group of Tyr_Z becomes very acidic upon oxidation and loses its proton to a nearby base, most probably the His190 that is in hydrogen-bonding contact (Diner & Rappaport 2002; Ferreira *et al.* 2004; Loll *et al.* 2005). The proton-coupled electron transfer (PCET) from Tyr_Z has been much studied and debated regarding the nature of the electron–proton coupling and its influence on the reaction rate and mechanism under different conditions (Tommos & Babcock 2000; Diner & Rappaport 2002; Diner *et al.* 2004; Renger 2004). The following reduction of the Tyr_Z radical by the Mn₄Ca cluster is also a PCET reaction, and thus the PCET of Tyr_Z may have direct mechanistic implications for the water oxidation.

From this section, it is clear that the concept of coupled electron-transfer reactions is vital for the function of PSII. While there have been numerous synthetic molecular systems shown to undergo light-induced electron transfer on the single-electron level,

very few have displayed accumulative electron transfer in a molecular unit or complex (Molnar *et al.* 1994; Heyduk & Nocera 2001; Huang *et al.* 2002, 2004; Konduri *et al.* 2002). Likewise, comparatively few synthetic molecular systems have shown light-induced PCET. Within the Swedish Consortium for Artificial Photosynthesis (CAP), we have focused on synthetic systems mimicking the donor side of PSII and made the first studies of light-induced electron transfer from tyrosine and synthetic manganese complexes to appended Ru(II)polypyridine complexes as photo-active units (Sun *et al.* 2001; Hammarström 2003; Lomoth *et al.* 2006). More recently, we have also made Fe₂ and linked Ru–Fe₂ complexes based on the active site structure of natural Fe-hydrogenases, achieving biomimetic hydrogen production (Ott *et al.* 2004a,b). In the present paper we briefly review some of our work relevant to the theme of coupled electron transfers in PSII.

2. RESULTS AND DISCUSSION

(a) Accumulative electron transfer from manganese complexes

At least two problems arise in accumulative electron transfer from a molecular unit or complex, which are not relevant for charge separation on the single-electron level. One is thermodynamic and is due to the build-up of charge on the complex. This makes it increasingly difficult to remove the next electron, unless each electron transfer is coupled to a charge-compensation mechanism. The other problem is kinetic and is due to the partly oxidized complex being able to act as both an electron donor and an acceptor to the excited state of the photo-active unit. As an illustration of this point, the Mn₄Ca cluster of PSII in the states S₁ to S₃ is thermodynamically very able to accept an electron from the excited *P₆₈₀, which would lead to an electron flow in a counterproductive direction. To avoid this reaction, the electron transfer steps in the productive direction must be more rapid to compete kinetically. The importance of both these points is illustrated below.

(i) Charge compensation in accumulative electron transfer

We have previously shown the light-induced accumulative oxidation of a manganese dimer linked to a photo-active Ru(II)(bpy)₃ complex (bpy is 2,2'-bipyridine, figure 2; Huang *et al.* 2002, 2004). Upon successive laser flash excitations, electrons were transferred from the excited Ru unit to the external, irreversible acceptor [Co(NH₃)Cl]²⁺, and the photo-oxidized Ru(III) oxidized the manganese dimer from the Mn₂(II,II) to the Mn₂(III,IV) state. This was initially surprising, as only two oxidation steps of the manganese complex were found electrochemically: Mn₂(II,II) → Mn₂(II,III) → Mn₂(III,III), both occurring below the Ru^{3+/2+} redox potential. We could later show, however, that the presence of water in the photoreactions led to a charge-compensating ligand exchange of the acetates for oxo ligands. A thorough study by Fourier-transform infrared (FTIR) spectroelectrochemistry and electrospray ionisation- (ESI-) mass spectrometry (Eilers *et al.* 2005) and extended X-ray absorption fine structure (EXAFS; Magnuson *et al.* 2006) led us to the following, somewhat

Figure 2. (a) A Ru–Mn₂ complex showing light-induced accumulative electron transfer from the Mn₂ to the photo-oxidized Ru unit. (b) A simplified scheme of the ligand exchange and proton release pattern upon oxidation of the Mn₂ complex in the presence of water ((i) less than 10% water, (ii) 90% water), as deduced from FTIR and ESI-MS spectroelectrochemistry.

simplified scheme (Lomoth *et al.* 2006): at low water concentration (less than or equal to 10%) and on short time scales (less than 30 s), ligand exchange occurs predominantly in the Mn₂(III, III) state. In 90% water instead, essentially all acetates have already dissociated in the Mn₂(II,II) state.

The ligand-exchanged species in the Mn₂(II,II) and Mn₂(II,III) state inferred from the FTIR and mass spectrometry data (Eilers *et al.* 2005) have water and hydroxo ligands, and they do not carry a lower charge than the corresponding states with acetate ligands. This may seem surprising, as charge compensation was evoked to explain the facilitated oxidation. However, the exchange for water ligands opens the possibility for a proton-coupled oxidation, in which the oxidized Mn₂(III,III) and Mn₂(III,IV) states produced are stabilized by a charge-compensating proton release. Indeed, the data suggest the release of about one, one and (at least) two equivalents of protons upon oxidation

of the Mn₂(II,II), Mn₂(II,III) and Mn₂(III,III) states, respectively. Also, the Mn₂(III,IV) complex detected by ESI-mass spectrometry appeared with two fully deprotonated, water-derived oxo ligands and has thus the same charge as the bis-acetato Mn₂(II,III) complex. Interestingly, our data indicate that the electrochemical potential required to produce the di-oxo Mn₂(III,IV) state in the presence of 10% water is just above that required to generate the bis-acetato Mn₂(II,III) state in neat acetonitrile. Although we could not determine the formal potentials, this would imply a compression of three oxidation steps within a narrow potential range of probably less than 0.15 V, thanks to a charge-compensating ligand exchange and proton-coupled oxidation. These reactions mimic the important stepwise oxidation of the manganese cluster of PSII, which is coupled to a charge-compensating deprotonation, presumably of water-derived ligands (Junge *et al.* 2002; Dau & Haumann 2006).

The FTIR spectroelectrochemistry data in dry acetonitrile (Eilers *et al.* 2005), when the acetates remain coordinated, may serve as references for IR-spectral changes expected for oxidation of carboxylate-bridged di-manganese units of the Mn_4Ca cluster of PSII. For the $\text{Mn}_2(\text{bpmp})(\text{Ac})_2$ complex (i.e. the same complex as in figure 2 but not linked to a Ru complex), the asymmetric stretch wavenumber changes as little as from 1594 cm^{-1} ($\text{Mn}_2^{\text{II,II}}$) to 1592 cm^{-1} ($\text{Mn}_2^{\text{II,III}}$) to 1586 cm^{-1} ($\text{Mn}_2^{\text{III,III}}$) upon oxidation. The wavenumbers for the symmetric stretch changed somewhat more, from 1422 cm^{-1} ($\text{Mn}_2^{\text{II,II}}$) to 1390 cm^{-1} ($\text{Mn}_2^{\text{II,III}}$) to 1384 cm^{-1} ($\text{Mn}_2^{\text{III,III}}$), but were broader and overlapped more with other absorption peaks. Thus, each peak shifts 6 cm^{-1} or less in most cases, which would be very difficult to detect in FTIR difference spectra of PSII. More comparisons with IR data from other model Mn-carboxylate complexes in different oxidation states up to Mn^{IV} would be necessary to say whether these small changes are typical or not and in order to guide the interpretation of FTIR data from PSII.

(ii) *Competing kinetics in accumulative electron transfer*

The excited state of the Ru unit of the $\text{Ru-Mn}_2(\text{II,II})$ complex in figure 2 has a lifetime of 110 ns (Sun *et al.* 2000). This allows efficient electron transfer to an acceptor to generate the Ru^{3+} state, and subsequent oxidation of the manganese dimer. When the manganese is already oxidized, however, the $\text{Mn}_2(\text{II,III})$ quenches the excited state so that its lifetime is much shorter (G. Eilers, L. Hammarström and R. Lomoth 2006, unpublished data). Although we have not elucidated the quenching mechanisms, the effect is that the shorter lifetime reduces the yield of electron transfer to the acceptor and is presumably one reason for the relatively low yield of manganese oxidation per flash (Huang *et al.* 2002).

In the corresponding Ru-Ru_2 complex, where the Ru dimer is isostructural with the Mn dimer above, we examined the quenching reactions in some detail in different oxidation states of the appended Ru dimer: $\text{Ru-Ru}_2(\text{II,II})$, $\text{Ru-Ru}_2(\text{II,III})$ and $\text{Ru-Ru}_2(\text{III,III})$ (Xu *et al.* 2005). In all states the excited state lifetime was very short, on the sub-nano-second time scale, owing to quenching reactions. For $\text{Ru-Ru}_2(\text{II,II})$, we concluded that the main quenching mechanism was exchange energy transfer, while in the higher oxidation states we could not discriminate between exchange energy transfer and electron transfer from the excited state to the Ru dimer. Nevertheless, irrespective of which mechanism is responsible for quenching, the net result is not productive for photo-oxidation of the Ru dimer, and the short excited state lifetime makes it difficult to obtain efficient electron transfer to an external acceptor. Instead we managed to increase the excited state lifetime by one order of magnitude by synthetically manipulating the remote ligands of the photo-active $\text{Ru}(\text{II})$ unit to localize the excited state (a metal-to-ligand charge-transfer state) away from the appended dimer and thus decrease the quenching rate. Using this approach, we have previously decreased the quenching in a Ru-Mn complex up to a factor of 600 (Abrahamsson *et al.* 2002). Importantly, the rate of the desired electron transfer to the photo-oxidized

Ru unit in the subsequent reactions was not decreased, because the electron is transferred to a metal-based orbital coupled via the same linking ligand.

These results show that unwanted quenching reactions of the photosensitizer might be very rapid and thus a real problem for accumulative electron transfer, also when the units are at approximately 15 Å distance. Nevertheless, the approach to localize the excited state away from the quenching Mn_2 or Ru_2 unit, while the 'hole' on the oxidized photosensitizer is still on the Ru metal, shows some promise. Note that it may also be analogous to the situation in PSII, for which a different distribution of the $^*\text{P}_{680}$ and P_{680}^+ states over the four central chlorophylls is discussed (Dekker & van Grondelle 2000; Renger & Holzwarth 2005).

(b) *Long-lived charge separation in a manganese-based triad*

By attaching two naphthalene diimide (NDI) electron-acceptor units to the Ru-Mn_2 complex above, we obtained the first manganese-based charge separation triad (figure 3; Borgström *et al.* 2005). Excitation of the Ru unit lead to rapid ($\tau = 40\text{ ns}$) electron transfer to the acceptor and oxidation of $\text{Mn}_2(\text{II,II})$. The charge-separated state had an average lifetime of $600\text{ }\mu\text{s}$ at room temperature, which is already unusually good for triad systems. By performing the experiments at 140 K instead (in fluid butyronitrile), the charge separation lifetime was dramatically increased to approximately 0.5 s , which is on the same time scale as charge recombination in photosynthetic reaction centres. We could detect both the oxidized donor and the reduced acceptor, and follow their recombination in a one-to-one ratio. The reaction was also reversible and we could run the triad through at least five charge-separation cycles without any detectable change in reactivity. This shows that it is a genuine charge-separated state of the triad.

The strong temperature dependence of the charge recombination process was analysed by Marcus theory (Marcus & Sutin 1985)

$$\ln(k_{\text{ET}}T^{1/2}) = \ln A - (\Delta G^0 + \lambda)^2/4\lambda RT, \quad (2.1)$$

where k_{ET} is the observed electron transfer rate constant; λ is the reorganization energy for electron transfer; A is a pre-exponential factor; and the other symbols have their conventional meanings. From the slope of the plot of $\ln(k_{\text{ET}}T^{1/2})$ versus $1/T$, and the value of $\Delta G^0 = -1.07\text{ eV}$, we derived a reorganization energy $\lambda = 2.0\text{ eV}$. This is twice as high as typically observed for electron transfer in polar media and implies a large inner reorganization energy of the manganese complex. By analysing the crystal structures of the $\text{Mn}_2(\text{II,II})$ and $\text{Mn}_2(\text{II,III})$ dimers, with reasonable assumptions for the bond force constants, we found that an inner reorganization energy in the order of 1.0 eV is reasonable. The bond-length changes are mainly a shortening of all the Mn-ligand bonds (also along the Jahn-Teller axis of $\text{Mn}(\text{III})$) of the manganese that is oxidized. This supports the experimental value, which includes an additional approximately 1.0 eV of solvent reorganization energy.

Figure 3. (a) A Mn₂-containing triad showing a very long-lived charge separation, with a lifetime of 600 μ s at room temperature and approximately 0.5 s in 140 K fluid butyronitrile solution. (b(i)) The diagram shows that the rate of back electron transfer (BET) in our triad lies far down in the Marcus normal region ($-\Delta G^0 < \lambda$) in spite of the large driving force, which explains the strong temperature dependence. (ii) Marcus free energy parabolas relevant for the BET. The dashed parabola represents a typical system with moderate reorganization energy for BET that lies in the Marcus inverted region.

Conventional wisdom concerning long-lived charge separation is that the recombination reaction should be in the Marcus inverted region, where the driving force ($-\Delta G^0$) is larger than the reorganization energy. This combines a reasonable energy content with a somewhat activated, and thus slow, recombination (figure 3). Interestingly, our results show long-lived charge separation in a different regime, in which the energy content is also high (approx. 1.0 eV) but the activated, slow recombination lies in the Marcus normal region. Because electron transfer from manganese is often accompanied by a large reorganization energy, it is a slow donor (Abrahamsson *et al.* 2002), but once it is oxidized this intrinsic property helps to maintain a long-lived charge separation. A

long-lived charge-separated state may allow for further light-induced charge separation and accumulative electron transfer, which we are currently exploring in this type of triad system.

(c) Proton-coupled electron transfer from tyrosine and tryptophan

As a model for PCET from tyrosine, we studied the intramolecular PCET of the Ru(bpy)₃-tyrosine complex of figure 4 (Sjödín *et al.* 2000, 2002, 2004). The Ru unit was photo-oxidized to Ru(III) by a laser flash in the presence of an external electron acceptor (methylviologen, [Ru(NH₃)₆]³⁺ or [Co(NH₃)Cl]²⁺). The subsequent PCET from the tyrosine is bidirectional, meaning that the electron goes to Ru(III) and

Figure 4. The rate constant of tyrosine oxidation as a function of pH for the $\text{Ru}(\text{bpy})_3$ -tyrosine complex (Sjödín *et al.* 2000). The solid line is a fit to a Marcus equation (equation (2.1)), with a decrease in ΔG^0 of 59 meV per pH unit and λ determined in independent experiments, while the dashed line is a guide for the eye. In a region around $\text{pH}=10$ the kinetics were biphasic.

the proton goes to the external aqueous solution. Bidirectional PCET reactions are often found in radical enzyme systems such as PSII, but more rarely in model systems (Chang *et al.* 2004; Mayer & Rhile 2004). The figure shows the pH dependence of the PCET rate constant. At $\text{pH}>10$ the tyrosine was already deprotonated and a rapid electron transfer was observed. At $\text{pH}<10$ a pH-dependent rate was observed, a pH dependence of a kind that had not been identified before.

The stepwise PCET mechanisms—proton transfer followed by electron transfer (PTET) and electron transfer followed by proton transfer (ETPT)—would not show this pH dependence. Based on well-established models and considerations, the limiting rate constant for proton transfer to H_2O , OH^- or the base form of the buffer are all too slow to explain the observed rates by any PTET mechanism (Sjödín *et al.* 2004, 2005). They would also give a slope of either 0 or 1 in figure 4, which is different from the slope of approximately 0.4 we observed. Instead we assigned this to a concerted electron–proton transfer (CEP) mechanism, in which the reaction coordinates for electron and proton are evolved to a common transition state. The assignment to a CEP mechanism was supported by a significant kinetic deuterium isotope effect and an activation energy that is larger than expected for a pure electron transfer.

As the tyrosine changes pK_a from 10 to -2 upon oxidation, the driving force for the overall PCET reaction increases with 59 meV per pH unit. We found that the rate followed the same dependence on pH, and thus on driving force, as that expected from the Marcus

theory for pure electron transfer. This is in contrast to the previously accepted idea that a CEP with proton release to the aqueous solution should not show pH dependence (Krishtalik 2003). Our finding is thus new, but a physical model that connects the microscopic PCET events to the macroscopic free energy dependence on pH, due to dilution of the released proton, remains to be developed to give a complete explanation of our results.

A bidirectional CEP reaction may be expected to have a larger reorganization energy than a pure electron transfer, owing to the additional solvent repolarization due to the proton release (Hammes-Schiffer & Iordanova 2004) and the internal bond-length changes in the phenolic group (Sjödín *et al.* 2005). Nevertheless, it can outcompete an ETPT mechanism because it uses all available free energy in a single reaction step. With the larger reorganization energy, the dependence of the rate on driving force in the Marcus normal region is less steep. Thus, by increasing the oxidant strength of the $\text{Ru}(\text{III})$ unit with ethyl ester substituents, we could favour ETPT and switch the dominating mechanism from CEP to the pH-independent ETPT (figure 5). The CEP mechanism could only compete at high pH values. In the $\text{Ru}(\text{bpy})_3$ -tryptophan complex, we changed instead the pK_a of the oxidized amino acid from -2 to approximately 4.7. This increased the driving force for the initial electron transfer step of ETPT, while the driving force for CEP was approximately the same as in the original $\text{Ru}(\text{bpy})_3$ -complex. Thus, we obtained a similar result as for the ester-substituted $\text{Ru}(\text{bpy})_3$ tyrosine: a pH-independent rate at low to neutral pH, followed by a pH-dependent rate at higher pH. As the deprotonation of the tryptophan radical is much slower than for the tyrosine radical, we could obtain direct spectroscopic evidence for a switch from a stepwise ETPT at neutral pH to a CEP reaction at high pH (figure 5). In the former case, the $\text{Trp}^{\cdot}\text{H}^+$ intermediate deprotonated with a time constant of 130 ns. This is expected for an Eigen acid with a pK_a of 4–5, and close to the value of 300 ns reported for $\text{Trp}^{\cdot}\text{H}^+$ deprotonation in DNA photolyase (Aubert *et al.* 2000). The competition between the ETPT and CEP mechanisms are governed by the higher driving force and higher reorganization energy for CEP. While ETPT is favoured by high oxidant strengths, CEP is energy conservative in that it uses all available free energy in a single reaction step. As low overall driving forces are typical for biological PCET reactions, one may predict that they most often follow a CEP rather than an ETPT mechanism.

Our original results in figure 4 showed strong kinetic similarities with data for Tyr_Z oxidation in Mn-depleted PSII, and we suggested that at $\text{pH}<7$, the latter also followed a CEP mechanism with proton release to the bulk. At higher pH, the Tyr_Z oxidation is faster and pH independent, however, owing to an internal hydrogen bond to His190. The kinetic difference compared with the low pH region—rate increase, activation energy and kinetic isotope effect—is less dramatic with this hydrogen bond than for complete deprotonation of the tyrosine as in our synthetic complex. We mimicked this situation in bimolecular oxidation of substituted phenols with internal hydrogen bonds to carboxylate groups on the

Figure 5. (a(i,ii)) The rate constant for amino acid oxidation as a function of pH for the Ru(bpy)₃-tryptophan and modified Ru(4,4'-CH₃COO-bpy)₂(bpy)-tyrosine complexes. Note that the rate scale is linear and not logarithmic as in figure 4. The solid lines are fits of the data to equation (2.1), consistent with a pH-dependent concerted reaction, with an additional constant term for the contribution from the stepwise ETPT mechanism. In (ii) the contributions from the two mechanisms are shown as dashed lines. In contrast to

phenol (Sjödín *et al.* 2006). The PCET still followed a CEP mechanism, but was now independent of pH because the proton was transferred to the carboxylate base. The driving force at neutral pH was smaller owing to the low pK_a values of the carboxylates. Still the rate was higher without the hydrogen bond, and the kinetic isotope effect was smaller. This may tentatively be ascribed to the combined effect of a smaller reorganization energy and better proton vibrational wave function overlap due to the stronger hydrogen bond (Hammes-Schiffer & Iordanova 2004; Sjödín *et al.* 2006). A phenol with a stronger hydrogen bond showed a larger rate increase and a smaller kinetic isotope effect than a phenol with a weaker hydrogen bond. This is an analogy to the case in PSII, where the Tyr_Z oxidation rate increases due to a hydrogen bond to His190 in the Mn-depleted system. In native PSII, this hydrogen bond is believed to be stronger and the rate then is even higher.

3. CONCLUSIONS

The paper has briefly summarized aspects of coupled electron transfer in the recent biomimetic efforts of the Swedish CAP. Just as extensive studies of single-electron transfer in model systems were pivotal for our understanding of single-electron transfer in biology, we now need model studies of different types of coupled electron-transfer reactions to understand the natural systems. Moreover, we need to master these reactions on a fundamental level in order to develop molecular systems for solar energy conversion by artificial photosynthesis.

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the complex of figure 4, the ETPT mechanism dominates up to a pH value of approximately 9, while the concerted mechanism dominates only at higher pH values. The kinetic component of the very rapid oxidation of the tyrosinate anion ($\tau < 20$ ns), present at pH values of approximately 10 and higher, is not shown. (b) Transient absorption spectra after photo-oxidation of Ru(bpy)₃-tryptophan with Ru(NH₃)₆³⁺, showing the spectra of the tryptophan radical. The spectra give direct evidence for a switch from a stepwise ETPT mechanism at intermediate pH to a CEP mechanism at high pH. At pH=3, which is below the pK_a value of the tryptophan radical ($pK_a=4.7$), the spectrum of the protonated radical is seen. At pH=8, the initial spectrum ($t=50$ ns; solid line) is from the initially formed protonated radical Trp[•]H⁺ that deprotonates with a time constant of approximately 130 ns to give the Trp[•] spectrum ($t=500$ ns; solid line with points). At pH=12, already the initial spectrum is that of the deprotonated Trp[•] radical ($t=50$ ns).

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Discussion

H. Dau (*Freie University, Berlin*). You suggested that in synthetic complexes a Mn-oxo group may be sufficient to form O₂ with an outer-sphere water molecule. I think that it also will be important to consider that groups of the Mn complex may be needed to accept the protons from the outer-sphere water. Can you comment on that?

L. Hammarström. Yes, proton-accepting bases may facilitate the deprotonation of water, and thus the water oxidation. We are working to introduce such groups in synthetic complexes.

A. Aukauloo (*University of Paris-Sud, France*). You proposed the formation of a Mn(V)=oxo species with the bomp ligand, but this ligand bears a phenol group. Do you think that this group stays in its normal oxidation state or gets oxidized to a phenoyl radical?

L. Hammarström. This part of our investigation is still at an early stage. As we presently see the same reaction signature with a range of complexes as reported for Mn₂(tpy)₂(H₂O)₂, i.e. formation of ¹⁶O¹⁸O with oxone in H₂¹⁸O, I suggested that the proposed mechanism for water oxidation involving a Mn(V)=O is more general to a variety of Mn-complex structures than presently believed, or that the mechanism is different from what has been proposed. Thus, Mn(V) may well never be involved in the bomp complex.

V. Pecoraro (*University of Michigan, USA*). It is likely that the *t*BuOOH reactions generating O₂ are going through *t*BuOOO*t*Bu giving *t*BuO[•] + ¹O₂.

L. Hammarström. We did not look for ¹O₂ as our isotope results already showed that this oxidant was unsuitable for our purpose. The oxygen seems to come entirely from manganese-catalysed degradation of *t*BuOOH.