

A Quantum Chemical Study of Hydrogen Abstraction from Manganese-Coordinated Water by a Tyrosyl Radical: A Model for Water Oxidation in Photosystem II

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Abstract: Recently, water oxidation in photosystem II was proposed to involve direct abstraction of hydrogen atoms from water molecules terminally ligated to manganese ions in the oxygen-evolving complex by the oxidized tyrosine radical, Tyr_Z[•]. This model is tested here by performing quantum chemical calculations. An empirically parametrized hybrid density functional method is used, and both monomeric and dimeric manganese model systems are studied. It is found that, by coordination to a manganese center, the first O–H bond strength of water is lowered from 113.4 to 84.3 kcal/mol. This O–H bond strength is only 2.8 kcal/mol stronger than that in tyrosine. Using an extended basis set, we find that this difference decreases still further. The second hydrogen abstraction energy is quite similar. Since thermoneutrality in the reaction (or a weak exothermicity) is a requirement for the hydrogen abstraction model, the present calculations support this model. Possible functions of a coordinated chloride and a nearby calcium complex are suggested. Five- or six-coordination and ferro- or antiferromagnetic spin couplings of the manganese centers are discussed.

Introduction

Photosystem II (PSII) is a large enzyme complex located in the thylakoid membranes in green plants, algae, and cyanobacteria.^{1–4} PSII is a key enzyme in the biosphere in that it catalyzes the light-driven electron transfer from water to the membrane bound electron carrier plastoquinone. In the process, two molecules of water are oxidized to molecular oxygen in a complex cyclic reaction known as the Kok or S-state cycle. Thus, the water-oxidizing complex in PSII provides the photosynthetic organisms with an unlimited source of electrons.

PSII (Figure 1) is composed of more than 20 protein subunits.^{1,4,5} Two of these, the D1 and D2 proteins, form a heterodimer that constitutes the PSII reaction center. The heterodimer carries most of the redox components involved in the light-driven electron transfer reactions while many of the other subunits are involved in the absorption of light through bound chlorophyll molecules or have regulatory functions. It is generally thought that the D1/D2 heterodimer also binds the Mn cluster that catalyzes the oxidation of water. This is comprised of four Mn ions that are held together by di- μ -oxo bridges and carboxylato bridges, and a tetrameric structure was

recently proposed mainly on the basis of EXAFS and EPR data.^{6–8} The inset in Figure 1 shows a slightly modified version of the structure proposed by Dau et al.⁶ that incorporates a second histidine ligand to one of the Mn ions in the cluster.⁹ The valence states of the Mn ions are known to be Mn(III) or higher in the dark-stable (S₁) state,^{10–13} and it is generally thought that the oxidation of water involves successive oxidations of the Mn cluster.^{2,3,14} However, at present, alternative mechanisms in which one or several steps involve radical formation cannot be excluded.

After the absorption of a light quantum, an electron is rapidly ejected from the primary electron donor P₆₈₀ (a dimer or multimer of chlorophylls) to the electron acceptor system, Figure 1. As shown in Figure 2, the oxidized donor P₆₈₀⁺ is rapidly reduced (in nanoseconds) by a nearby (10–15 Å)^{15,16} tyrosyl residue denoted tyrosine_Z (Tyr161 in the D1 protein^{17,18}), which

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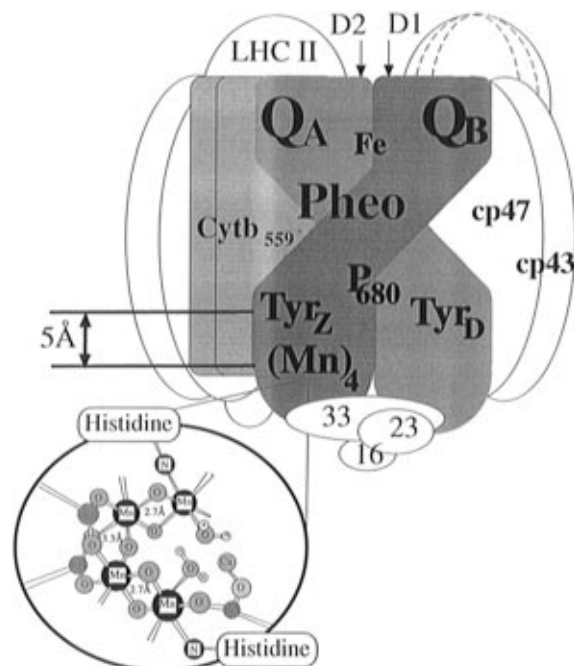


Figure 1. Schematic model of the photosystem II reaction center. The figure shows the D1/D2 heterodimeric reaction center surrounded by some other PSII protein subunits including cytochrome b_{559} , three proteins of molecular masses 16, 23, and 33 kDa that are known to be involved in the formation of the water-oxidizing complex, and the chlorophyll-containing proteins LHC II, CP43, and CP47. The figure also shows the redox components involved in the photochemistry in PSII. P_{680} is the primary donor, and Pheo, QA, and QB constitute the electron acceptor complex, while Tyr_Z and the Mn_4 cluster are involved in the oxidation of water. Enlarged is the suggested structure of the Mn cluster which is based primarily on EXAFS data.^{6,7} Two histidine ligands to Mn are pointed out on the basis of recent chemical modification studies.⁹ A Ca ion is placed bridged via a carboxylate ligand as suggested in ref 10b. The approximate location of the Mn cluster close to Tyr_Z is also indicated.

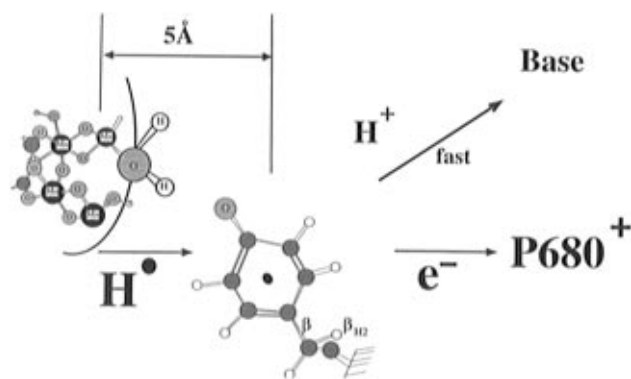


Figure 2. Proposed dual function of Tyr_Z . It rapidly donates an electron to the photooxidized primary donor P_{680}^+ . Simultaneously the proton is released from the tyrosine, thereby forming a deprotonated oxidized radical. This is suggested to abstract a hydrogen atom from a metal-coordinated water molecule at the nearby Mn cluster.

functions as an interface to the Mn cluster where the water is ultimately oxidized.^{19,20}

The mechanism for oxidation of the manganese cluster (OEC) in PSII has earlier been assumed to occur by simple electron-transfer steps to the nearby oxidized Tyr_Z^{ox} radical.^{2,14,21} For such electron transfer processes in enzymes, much is known

about the requirements of the system necessary for optimal performance.²² Factors affecting the efficiency are, for example, the hydrophobic character of the specific site as well as the rigidity of the redox systems involved. Recent results obtained from EPR studies of the Tyr_Z radical in PSII are reported to be inconsistent with a pure electron transfer function of this radical.^{19,20} In parallel with a few other redox enzymes, where tyrosyl radicals also appear to have important functions, it was suggested that Tyr_Z acts as hydrogen abstractor, mediating electron transfer between the manganese cluster and the photo-oxidized chlorophyll in photosystem II. The mode of action was suggested to be oxidation of the manganese cluster by abstraction of hydrogen atoms from coordinated water or hydroxide.^{19,20} A similar atom abstraction function for Tyr_Z was postulated independently from other electron magnetic resonance results that showed that Tyr_Z and the manganese cluster were in close physical proximity.²³ The structure of the $Tyr_Z/(Mn)_4$ site, the binding location for substrate water, and the reaction chemistry between the tyrosine and the ligated water envisioned in the atom abstraction model are summarized in Figure 2. Intuitively, this type of abstraction by tyrosine may appear unfavorable since the phenolic O–H bond in tyrosine is weaker than the corresponding bond in water by about 30 kcal/mol. We therefore decided to investigate the influence on the O–H bond strength in water of coordination to manganese by quantum chemical methods.

In our work, several Mn structures of increasing complexity were used to model the natural Mn complex. In the simplest structures only one manganese atom was included, while the more realistic structures included two manganese atoms. For each one of these structures, the two O–H bond strengths were evaluated for a water molecule coordinated to a manganese atom. For these bond strengths, it is quite reasonable to assume that it is the directly bonding manganese atom that will be by far the more important. The second manganese atom, connected to the first by μ -oxo bridges, will at most be of second-order importance. This assumption is confirmed by the present calculations. The third and the fourth manganese atoms in structure 1 can therefore safely be left out in calculating bond dissociation energies for the reaction steps studied. The situation is, of course, different for the reaction where molecular oxygen is eventually formed, but this step will not be discussed in the present paper. The geometries of the manganese complexes were fully optimized by using a hybrid density functional theory (DFT) method termed B3LYP,²⁴ which is described in the Computational Details. Rather small basis sets were used for the geometry optimization. At the optimized geometries, the energy was evaluated, again at the B3LYP level, but now using a much larger basis set.

Computational Details

The calculations were performed in two steps. First, an optimization of the geometry was performed using B3LYP, a density functional

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theory (DFT) type of calculation based on hybrid functionals, and double- ζ basis sets. In the second step the energy was evaluated in the optimized geometry using basis sets with polarization functions. The final energy evaluation was also performed at the B3LYP level. All calculations were performed using the GAUSSIAN-94 program.²⁵

The present DFT calculations were made using the empirically parametrized B3LYP method.²⁴ The B3LYP functional can be written as

$$F^{\text{B3LYP}} = (1 - A)F_x^{\text{Slater}} + AF_x^{\text{HF}} + BF_x^{\text{Becke}} + CF_c^{\text{LYP}} + (1 - C)F_c^{\text{VWN}}$$

where F_x^{Slater} is the Slater exchange, F_x^{HF} is the Hartree–Fock exchange, F_x^{Becke} is the gradient part of the exchange functional of Becke,²⁴ F_c^{LYP} is the correlation functional of Lee, Yang, and Parr,²⁶ and F_c^{VWN} is the correlation functional of Vosko, Wilk, and Nusair.²⁷ A , B , and C are the coefficients determined by Becke²⁴ using a fit to experimental heats of formation. However, it should be noted that Becke did not use F_c^{VWN} and F_c^{LYP} in the expression above when the coefficients were determined, but used the correlation functionals of Perdew and Wang instead.²⁸ The B3LYP method actually used was suggested by Stevens et al.²⁹

The B3LYP energy calculations were made by using the large 6-311+G(1d,1p) basis sets in the Gaussian-94 program. This basis set has one set of polarization functions on all atoms, and also diffuse functions, which are found to be important when interactions with oxygen-containing systems like water are studied. All results below will refer to the use of this basis set unless otherwise specified. For some of the manganese monomer calculations the still larger 6-311+G(2d,2p) basis set with two polarization functions per atom was used. This basis set will be referred to as the extended basis set. In the geometry optimizations a much smaller basis set, the LANL2DZ set of the Gaussian-94 program, was used. For the manganese atom this means that a nonrelativistic ECP according to Hay and Wadt³⁰ was used. The valence basis set used in connection with this is essentially of double- ζ quality including a diffuse 3d function. The rest of the atoms are described by standard double- ζ basis sets.

Zero-point vibrational effects on the O–H bond strengths were calculated at the B3LYP level for water, phenol, and the monomeric manganese complexes included in model 5. The phenol value was used for tyrosine, and the values for model complex 5 were used for all the other manganese models. These calculations were performed with an all electron basis of double- ζ quality by using the Wachters basis³¹ for manganese.

Results and Discussion

Structure 1 proposed for the Mn₄ cluster in PSII⁷ shown in Figure 3 does not assign definite oxidation states for the manganese atoms. In the present study, we have chosen to give the manganese that coordinates the reacting water molecule a starting oxidation state of III, which is one of the possibilities

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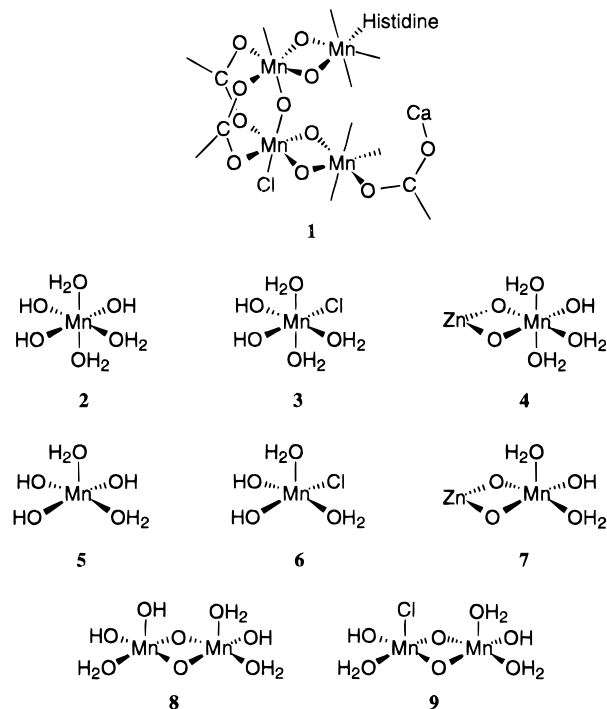


Figure 3. Proposed structure of the Mn₄ cluster in PSII, 1, and models of the Mn₄ cluster used in the calculations, 2–9.

Table 1. O–H Bond Strengths (kcal/mol)

complex	first H	second H	complex	first H	second H
	abstraction	abstraction		abstraction	abstraction
2	78.0		8	84.3	85.0
3	83.2		9	84.9	85.8
4	77.4		tyrosine	81.5	
5	83.3	83.5	phenol	82.4	
6	89.0	96.8	H ₂ O	113.4	
7	80.9	87.2			

consistent with experimental data.^{10–13} Other possibilities for the manganese oxidation states are under investigation. It is, for example, quite possible that the two manganese atoms that coordinate reacting water molecules might start with different oxidation states. This question is not addressed in the present model study, which is aimed primarily at providing an initial assessment of the energetic feasibility of a H atom abstraction model for water splitting.

The calculations of the O–H bond strengths of a water molecule coordinated to a Mn(III) center were performed for several different models for the proposed biological structure 1 (Figure 3) with increasing complexity. The model structures are shown schematically in Figure 3, 2–9, in the order they were actually used in the computations. As can be seen only 2–4 have the initially expected six-coordinated manganese centers. It turned out, as will be described below, that the Mn(III) center tends to lose one water ligand to the second coordination sphere. Even though it is possible to force the geometry optimization to converge to a structure with six directly coordinated ligands, our calculations show that this structure is always substantially higher in energy than the five-coordinated structures.

The first value of importance for the present study is the O–H bond strength in tyrosine. The calculations give a value of 81.5 kcal/mol for this bond strength, which can be compared to the calculated value of 113.4 kcal/mol for the O–H bond strength in water and of 82.4 kcal/mol in phenol, Table 1. The corresponding values that were obtained by using the extended basis set with two sets of polarization functions are 82.2, 114.4,

and 83.1 kcal/mol, respectively. The experimental values are 118.0 kcal/mol for water³² and 86.5 ± 2 kcal/mol for phenol,³³ which shows that the calculations seem to systematically underestimate these bond strengths by about 4 kcal/mol. For the present reaction steps, a systematic underestimation is not very serious since in the reactions one O–H bond is broken and one is formed, which means that the errors will cancel.

The first set of calculations on metal-coordinated water were performed for a complex with only one manganese atom. Starting out with a six-coordinated Mn(III) complex, **2**, the geometry optimization converged to a structure with only five ligands directly coordinated to manganese and with one water ligand in the second coordination shell, as mentioned above. A second attempt to actually keep six coordinated ligands was successful but led to a higher energy. Comparing the stability of these two structures, we found that the six-coordinated structure is 7.4 kcal/mol higher in energy than the five-coordinated structure with one water molecule in the second coordination sphere. The energy difference is even larger for the small basis set used in the geometry optimizations. This is admittedly a somewhat surprising result, but the conclusion from the calculations, that manganese(III) is only five-coordinated when water and hydroxide constitute the ligand set, is quite definite on this point. Several examples of five-coordinated Mn(III) complexes can be found in the literature,³⁴ and also in PSII five-coordination for Mn(III) ions has been indicated by EPR spectroscopy.³⁵

The preference for five-coordination can be rationalized as an extreme case of Jahn–Teller (JT) distortions. The bond elongations along the JT axis are so large that a water ligand is actually lost. Large JT distortions can be expected for this type of Mn(III) system with one e_g -electron. Similar distortions were recently seen also for Fe(IV) systems in a theoretical study of methane monooxygenase (MMO).³⁶ The final Mn(V) systems also strongly prefer to be five-coordinated, with a structure that is best described as a trigonal bipyramid. In this case the weak bonding of water can be described as a trans effect. In the intermediate Mn(IV) systems on the other hand, five- and six-coordinations are about equally favorable; i.e., starting with the five-coordinated complex, an extra water molecule binds with the same strength in the sixth position in the first coordination sphere as it does in the second coordination sphere, where it hydrogen bonds to the other ligands. Furthermore, for the five-coordinated Mn(IV) complex, a coordination geometry in which a hydroxide ligates opposite the empty position is strongly preferred, which is also a trans effect. A consequence of this is that it is energetically equivalent to abstract a H atom from a water molecule in either axial or equatorial positions in the Mn(III) complex, since the Mn(IV) complex rearranges to the same structure in the two cases. In this situation, where five-coordination is preferred for two of the oxidation states involved, and for the third oxidation state five- and six-coordinations are equivalent, it seems most reasonable to use five-coordinated models in all cases.

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In order to see if the preference for five-coordination is the result of a limitation of the mononuclear Mn model chosen as the initial target for computational analysis, a somewhat more realistic structure was used. In particular, it might be possible that the presence of μ -oxo bridges in the Mn complex could change the situation. Therefore, these bridges were included by using structure **4** where the second manganese center is modeled by a zinc atom. The zinc atom is here used as the simplest possible way to introduce the μ -oxo bridges. The geometry optimization again led to the result with the five-coordinated structure more stable than the six-coordinated structure. For the zinc complex **4**, the energy difference between the five- and the six-coordinated structures is 12.0 kcal/mol. Since this difference is even larger for the small basis set used in the geometry optimization, the geometry optimization was repeated with a larger basis set with all-electron basis sets for manganese and zinc and polarization functions on hydrogen. The energies (using the 6-311+G(1d,1p) basis set) in the resulting geometries led to an energy difference between the five- and the six-coordinated structures of 13.1 kcal/mol, which is quite similar to the result of 12.0 kcal/mol for the less accurate geometries. It can be added that, for the five-coordinated complexes, the bond strength of the water molecule in the second coordination sphere was also evaluated and a value of 15.5 kcal/mol was obtained. The corresponding result for the monomeric complex is very similar, 15.7 kcal/mol. On the basis of these results, we will focus on model complexes with only five ligands in the following.

It is interesting to note that five-coordination has recently been observed in the binuclear manganese cluster in arginase.³⁷ In that protein the binuclear manganese site is bridged by carboxylate groups and solvent water. One of the manganese atoms is six-coordinate (distorted octahedral), and the other is only five-coordinate (square pyramidal).

To investigate the reliability of the calculations further, two sets of calculations were performed. In one set, the coordination of Mn(II) was investigated. In these calculations, six-coordination of $\text{Mn}(\text{H}_2\text{O})_6^{2+}$ was compared to five-coordination, with the sixth water molecule hydrogen bonded to one or two of the water molecules coordinating directly to manganese. In agreement with the solution behavior of Mn(II) the calculations showed that the six-coordinated structure was the most stable one, but only by about 3 kcal/mol. In another set of calculations, the O–H bond strength in $\text{MnO}_3(\text{OH})^-$ was calculated, to be compared to an experimental value of 80 ± 3 kcal/mol obtained from a thermochemical cycle calculation based on the redox potential and pK_a values.³⁸ The calculated value for this O–H bond strength is 76.1 kcal/mol. It is 77.2 kcal/mol with the extended basis, both values in good agreement with the experimental value.

Before the results on bond dissociation energies for the monomeric manganese complexes are discussed, we comment on the electronic structure of these complexes. The starting complex for the calculation of the energetics of hydrogen atom abstractions is $\text{Mn}^{\text{III}}(\text{H}_2\text{O})_2(\text{OH})_3$, **5**. Two of the OH groups model μ -oxo bridges, the third OH group models a carboxylate anion (or a glutamate side chain), one of the water ligands models a lone-pair ligand such as imidazole (or a histidine ligand), and finally the fifth ligand is the actual water ligand of interest from which the hydrogen atoms are abstracted. The ground state of this Mn(III) complex is ^5A , and the unpaired electrons are almost completely located at the manganese atom.

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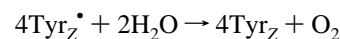
The adiabatic excitation energy to the 3A state is 32 kcal/mol when the small basis set without polarization functions is used. When a hydrogen atom is abstracted by the tyrosyl radical, the complex becomes $Mn^{IV}(H_2O)(OH)_4$. The ground state of this $Mn(IV)$ complex is 4A , as expected from the 5A ground state of **5** and the fact that a covalent bond is formed between manganese and the new OH group formed in the $Mn(IV)$ complex. Finally, when the second hydrogen atom is removed, the complex becomes $Mn^V(H_2O)(OH)_3O$. This complex has a 3A ground state with a double bond between manganese and the oxo oxygen. The 1A state is calculated to be 13.1 kcal/mol higher than the 3A ground state. The singlet state has a short Mn–O distance, 1.55 Å, indicating a triple Mn–O bond, and is in agreement with experimental results for diamagnetic $Mn(V)$ –oxo complexes (1.55–1.56 Å).^{34b,39} The triplet ground state obtained for the $Mn^V(H_2O)(OH)_3O$ complex might be considered surprising since many d^2 oxo complexes are observed to be diamagnetic. However, the order of the singlet and triplet states of d^2 oxo complexes depends on the other ligands, and it can be mentioned that calculations on a model of a porphyrin $Mn(V)$ –oxo complex gave a singlet ground state, with a vertical excitation energy of 32 kcal/mol, in agreement with experimental results on $Mn(V)$ –oxo porphyrin compounds.

The excitation energy to the high-spin 5A state from the 3A ground state of $Mn^V(H_2O)(OH)_3O$ is 24.0 kcal/mol from calculations in which the large basis set was used. In the high-spin state, the Mn=O double bond is partly broken and the oxygen atom carries one unpaired electron and can thus be considered as a radical. The spin population on this oxygen is 0.91. This finding, together with the rather low excitation energy, is probably quite significant for the final step in the photosynthetic oxygen evolution cycle, since it shows that formation of O_2 from complexes where oxygen radicals are present might be possible.

The Mulliken charges on manganese with the small basis set are +0.97 for the $Mn(III)$ complex, +0.96 for the $Mn(IV)$ complex, and finally +0.87 for the $Mn(V)$ complex. The charges on the water ligands vary between +0.1 and +0.2, and the charges on the hydroxyl ligands vary between –0.2 and –0.4. The oxo oxygen carries a charge of about –0.4. The populations in the 3d shell in the three complexes are 5.25, 5.27, and 5.38 electrons, respectively. These values are consequently very far away from the charges and 3d populations that are expected from literal interpretations of oxidation states. It is clear that some of this discrepancy between the conventional picture and the present results arises from the arbitrariness (and basis set dependence) of the Mulliken population analysis. However, it is also evident that, for example, the remarkable constancy of the 3d population is in part significant and implies a quite different picture of the electronic structure in these systems than the common ionic one. In practice, there is a tendency for the manganese atom in the complexes to stay in approximately the same electronic structure environment as that in the free atom (which has a $3d^54s^2$ ground state). To change the 3d population significantly is quite expensive, which is evident from the large energies involved when the number of 3d electrons is changed for the free atom.

The energy differences between the monomer model complexes **5** discussed above lead to the following O–H bond energies for a water ligand bound to the metal. The first O–H bond strength, going from the water ligand to a hydroxyl ligand, is 83.3 kcal/mol, and the second O–H bond strength, going

from the hydroxyl ligand to an oxo ligand, is 83.5 kcal/mol (see Table 1). With the extended basis set with two polarization functions, these values decrease to 82.6 and 82.3 kcal/mol, respectively. The first important conclusion from these values is that the effect of metal coordination on the water bond strengths is quite large, with a reduction of about 30 kcal/mol. This is precisely enough to make the abstraction reaction with the Tyr_Z radical possible. In fact, the abstraction reaction is almost perfectly thermoneutral, which at first seems like a strange coincidence. However, the overall catalytic process performed by the manganese complex in PSII, which is



is only 14.0 kcal/mol exothermic according to the present calculations. The experimental value, which we obtained by using phenol instead of tyrosine, is 22.7 ± 10 kcal/mol, where the large error bars mainly come from the 2 kcal/mol uncertainty of the experimental O–H bond strength in phenol.³³ It should also be noted that the exothermicity is obtained under the assumption of lack of hydrogen bonding in the above equation. Any energetic differences in hydrogen bonding before and after the reaction will change the exothermicity. Since the hydrogen bonding energies of the waters and the tyrosyl radicals can be expected to be larger than those for the tyrosines and the oxygen molecule, the actual exothermicity of the catalytic cycle in PSII will be even smaller. In PSII, the above reaction occurs in five steps in the S cycle, where the first four concern O–H abstractions and where O_2 is formed in the fifth step.^{2,3} If all these steps are to be exothermic, which is a reasonable requirement, then each step can be only weakly exothermic. Furthermore, the most demanding step is most certainly the final fifth step in which O_2 is formed, and this step might therefore require a larger exothermicity than the other steps. Therefore, almost thermoneutral O–H abstractions are, in fact, required, if the recently suggested mechanism involving tyrosyl abstractors is to be possible. The main conclusion from the monomer results is, therefore, that they strongly support the tyrosyl abstractor mechanism.

The next step in the modeling of the manganese cluster **1** is to introduce the μ -oxo bonds, which are generally thought to be present in the natural Mn cluster.^{6,7} This is initially done as in the complexes **4** and **7** by using a zinc atom. The introduction of the μ -oxo bonds has only a small effect on the bond strengths as seen in Table 1. The first O–H bond is changed from 78.8 to 77.4 kcal/mol for the six-coordinated complex **4** and from 83.3 to 80.9 kcal/mol for the five-coordinated complex **7**. The second O–H bond strength was only studied for complex **7**, and it is changed from 83.5 to 87.2 kcal/mol. The sum of the two O–H bond strengths is almost exactly the same in the zinc complex **7** as in the monomer complex **5**.

The use of a Mn dimer complex, **8**, represents the next higher level of modeling the actual manganese cluster **1**. The main computational problem of treating the complex **8** is, besides the size of the system, the treatment of spin. The spin is quite high on the manganese atoms, and experimentally, these spins are thought to be antiferromagnetically coupled, since the S_1 and S_3 states are EPR invisible. It is extremely difficult to control the convergence to this type of coupling between the metal spins. For example, antiferromagnetic coupling with two equal spins on the manganese atoms will produce a singlet state, which is only one of many possible singlet states. Instead, if the spins are ferromagnetically coupled, a uniquely defined wave function of high spin results, which is much easier to converge. In the following calculations, therefore, a significant advantage would be gained if ferromagnetic coupling could be used in

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the dimer models. A requirement for doing this is, of course, that this is a reasonable approximation to the real antiferromagnetic coupling situation. Fortunately, this turns out to be the case. For some of the manganese dimer complexes it was actually possible to converge both solutions, the ferromagnetic and the antiferromagnetic cases, and the energy differences between these solutions are small. For the $\text{Mn}^{\text{IV}}(\text{H}_2\text{O})(\text{OH})_2-\text{O}_2-\text{Mn}^{\text{IV}}(\text{H}_2\text{O})(\text{OH})_2$ complex, the energy difference between the high-spin ${}^7\text{A}$ state and the low-spin ${}^1\text{A}$ state is only 2.2 kcal/mol in calculations with the small basis set, with the singlet state being the lower energy state. Also, for the $\text{Mn}^{\text{V}}(\text{H}_2\text{O})(\text{OH})\text{O}-\text{O}_2-\text{Mn}^{\text{IV}}(\text{H}_2\text{O})(\text{OH})_2$ complex the energy difference between the high-spin ${}^6\text{A}$ state and the low-spin ${}^2\text{A}$ state is 1.8 kcal/mol, in favor of the doublet state. The similarity in the low-spin preference for these systems also means that the relative effect on the O–H bond strengths is even smaller and certainly without consequence for the present study. In this context it is interesting to note that the present hybrid DFT method (B3LYP) is able to reproduce the experimentally known preference for antiferromagnetic coupling between the manganese spins, even though the antiferromagnetic spin coupling is not strictly correct in this method. Apart from the difference in spin coupling the electronic structure differences between the high-spin and low-spin states are very small. One consequence of this small difference is that the optimized geometries for the two spin states are virtually identical. The conclusion from these comparisons is that, for the atom abstraction sequence considered in the computations presented here, it is quite satisfactory to use ferromagnetically coupled manganese dimers, which simplifies these studies considerably. As a final comment on the use of ferromagnetic coupling, it can be noted that a set of complete active space SCF (CASSCF)⁴⁰ calculations were also performed, since in this method the spin is correctly treated. However, since there is very little dynamical correlation at this level, the spin is much too delocalized over the ligands and the results therefore became unreliable. A treatment that goes significantly beyond the present B3LYP treatment in accuracy will thus require an effort that is far beyond the present possibilities.

For the natural Mn_4 complex reasonably good understanding of the Mn valence exists for the S_1 and S_2 states. Currently the preferred redox state of the Mn cluster in the S_1 state is $\text{Mn}^{\text{III}}_2\text{Mn}^{\text{IV}}_2$, which is derived mainly from XANES investigations,^{10–13} but it cannot be ruled out that the valence configuration instead is Mn^{III}_4 . On advancement to the S_2 state the Mn cluster is oxidized, resulting in either a $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}_3$ or a $\text{Mn}^{\text{III}}_3\text{Mn}^{\text{IV}}$ configuration, and presently there is little agreement in the literature on which configuration is most likely.^{2b,3,10–14} However, for the calculations, the most symmetrical choice for the second manganese atom is Mn^{IV} , which means that the initial water complex (in the calculations) will be in the $\text{Mn}^{\text{IV}}\text{Mn}^{\text{III}}$ state, more specifically $\text{Mn}^{\text{IV}}(\text{OH})_2(\text{H}_2\text{O})-\text{O}_2-\text{Mn}^{\text{III}}(\text{OH})(\text{H}_2\text{O})_2$ (Figure 4a). This state then would correspond to either the S_1 or the S_2 state, dependent on which of the assignments for the natural Mn cluster described above ultimately is found to be correct.

The ground state of the configuration in Figure 4a with ferromagnetic coupling is ${}^8\text{A}$. As a hydrogen atom is abstracted, the complex shifts to $\text{Mn}_2^{\text{IV,IV}}$, see Figure 4b, with ground state ${}^7\text{A}$, and after the final hydrogen abstraction, the complex is $\text{Mn}_2^{\text{IV,V}}$, see Figure 4c, with ground state ${}^6\text{A}$. The calculated bond strength is 84.3 kcal/mol for the first hydrogen and 85.0

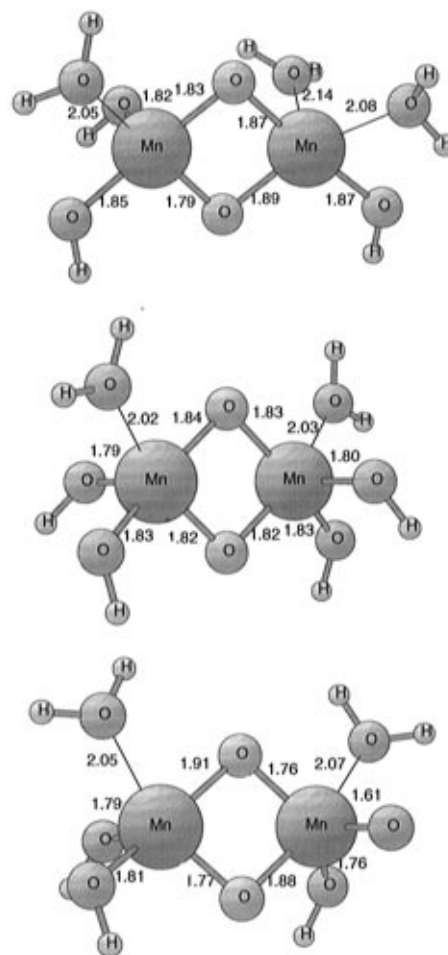


Figure 4. $\text{Mn}(\text{OH})_2(\text{H}_2\text{O})-\text{O}_2-\text{Mn}(\text{OH})(\text{H}_2\text{O})_2$ model used for the hydrogen abstraction calculations. The hydrogens are abstracted from the rightmost water molecule. (a, top) Starting water complex. The first O–H bond strength is 84.9 kcal/mol. (b, middle) Intermediate hydroxyl complex. The second O–H bond strength is 85.8 kcal/mol. (c, bottom) Final oxo complex.

kcal/mol for the second hydrogen, Table 1. The results are thus very close to the monomer results discussed above.

An interesting property of the manganese dimers is the radical character of the bridging oxygens. It is found that the spin delocalization on these oxygens is rather small, indicating low radical character. For the $\text{Mn}_2^{\text{IV,III}}$ dimer the spin populations are +0.03 and –0.04, for the $\text{Mn}_2^{\text{IV,IV}}$ dimer they are –0.01 and +0.17, and for the $\text{Mn}_2^{\text{IV,V}}$ dimer they are +0.02 and +0.02. This can be compared to recent calculations on a similar bis- $(\mu\text{-oxo})-\text{Fe}_2^{\text{IV,IV}}$ dimer, for which the spin populations on the bridging oxos are as high as +0.56 and +0.56.³⁶ The low radical character on these oxygens for the manganese dimers has certain chemical implications. It is, for example, not expected that these bridging oxygens will take part in any side reaction such as abstracting a hydrogen from another ligand or from a nearby amino acid. Furthermore, it does not seem likely that they will combine to form O_2 at any stage. A preliminary calculation places the $\text{Mn}(\text{III})-(\mu\text{-O}_2)-\text{Mn}(\text{III})$ peroxy complex 12.2 kcal/mol above the $\text{Mn}(\text{IV})-(\mu\text{-O})_2-\text{Mn}(\text{IV})$ bis- $(\mu\text{-oxo})$ complex. The mechanism for the O_2 evolution in PSII therefore probably does not involve bridging oxo collapse. (For a recent review where that mechanism is considered see ref 41.) The results of the present calculations support the idea that dioxygen bond formation occurs at terminal positions in the cluster.^{19,20}

(40) MOLCAS is a program package written by Andersson, K.; Fülscher, M. P.; Lindh, R.; Malmqvist, P.-Å.; Olsen, J.; Roos, B. O.; Sadlej, A. J.; Widmark, P.-O.; Blomberg, M. R. A.; Siegbahn, P. E. M., 1995.

(41) Yachandra, V. K.; Sauer, K.; Klein, M. P. *Chem. Rev.* 1996, 96, 2927.

In the calculations on the manganese dimer complexes, hydrogen bonds between the ligands sometimes occurred, and it was quite important to keep the hydrogen bonding situation as similar as possible before and after the hydrogen abstractions. In several calculations, the geometry converged to a complex with hydrogen bonds between a ligand on one manganese center and a ligand on the other manganese center. If these hydrogen bonds are the same before and after the abstraction, the abstraction energy is hardly affected. However, if the hydrogen bonding is present before and not after the abstraction, the abstraction energy can easily change by 5–7 kcal/mol. It is difficult to control the geometry convergence completely, and several attempts therefore had to be made before consistent results were obtained. It is probably true that the choice of hydroxyl and water ligands in the present models exaggerates the possibility for hydrogen bond formation. In the real complex, ligands such as glutamate lack OH groups, which should reduce the possibilities to form hydrogen bonds. However, some hydrogen bonding is possible also in the real complex, and it should therefore be added that the present hydrogen abstraction model does not exclude the presence of hydrogen bonding. The assumption made in the present model is instead that, if hydrogen bonds are present, they should not be significantly changed during the hydrogen abstractions. To explicitly study the effects of hydrogen bonding in OEC, the ligands that actually occur in the real complex have to be used in the calculations.

A few model calculations on other important features in PSII were also made. These calculations indicate that additional investigations are required to obtain a complete understanding of these hydrogen abstraction reactions. Although these investigations are in progress, some preliminary results will be reported here. There is a wealth of information indicating the importance of Cl^- and Ca^{2+} in the natural water oxidation reaction (see refs 2b and 3 and references therein). Both cofactors are thought to bind close to or directly to the Mn cluster, see Figure 1 for the suggested position of calcium, and they are known to affect certain redox steps in the water oxidation reaction. A widely accepted opinion is that they somehow regulate the Mn cluster to avoid erroneous partial oxidation of water that may form dangerous oxygen radicals or hydrogen peroxide.^{3,42} In the first modification of the models considered above, a hydroxyl ligand was replaced by a chloride ligand. For the monomeric case, model **3** is obtained for the six-coordinated case and **6** for the five-coordinated case. As seen in Table 1, the presence of a chloride ligand increases the hydrogen abstraction energy substantially, by 5–6 kcal/mol for the first abstraction and even more for the second abstraction. The increase of the first hydrogen abstraction energy on exchange of a hydroxyl ligand for a chloride is due to, in equal amounts, an increase in the Mn–OH₂ bond strength of the coordinated water molecule before the reaction and a decrease in the Mn–OH bond strength of the OH group formed after the reaction. The increase in the Mn–OH₂ bond strength is due to a local charge rearrangement caused by the chloride ligand, and the decreased Mn–OH bond strength is due to a slight increase in spin density on the oxygen caused by the chloride ligand. The large increase in the second hydrogen abstraction energy on exchange of the hydroxyl ligand for a chloride is due to a very large decrease in the Mn–O bond strength in the final oxo compound. This can be seen as a large involvement of an open-shell electronic structure corresponding to a partial breaking of the Mn–O double bond, leading to a high spin density on the oxygen for the chloride case.

Exchanging a hydroxyl ligand with a chloride ligand was also investigated for the dimer model **9**, where the ligand exchange is done for the secondary manganese center in the dimer. As seen in Table 1, and as expected, this ligand exchange has only a very small effect on the hydrogen abstraction energy from a water on the other manganese center.

The water and hydroxyl ligands used in the compounds discussed above only mimic the actual ligands, which are dominated by carboxylic side chains and histidine ligands. Accordingly, we tested the effects of exchanging these ligands. For the monomeric case, replacement of one water ligand by an imidazole increased the hydrogen abstraction energies, but only by 1.5–2.0 kcal/mol.

In contrast, a much more significant effect was obtained when a hydroxyl ligand was replaced by a formate ligand to mimic a carboxylate side chain. In this case the first hydrogen abstraction is only affected to a minor extent, while the second hydrogen abstraction energy is increased by a significant 11.6 kcal/mol for the dimer model to a value of 96.6 kcal/mol. This is substantially higher than the O–H bond strength in tyrosine. For the monomer, a similar effect of +11.1 kcal/mol was obtained, leading to a bond strength of 94.6 kcal/mol for the second hydrogen abstraction. The reason for this increase is that a lone pair on the noncoordinating oxygen atom of the formate ligand in the monodentate complex is attracted to the manganese center, even though it is in the second coordination sphere. A value as high as 95–97 kcal/mol will violate the present hydrogen abstraction model to some extent. The attraction between the second oxygen in the formate and the manganese center therefore has to be blocked in some way. An interesting possibility is that a calcium ion, known to be present in this region,^{3,6,42} could diminish or abolish the interaction. A test calculation was performed for the monomer model with a CaCl_2 complex present as well. This leads to the expected blocking, and the second hydrogen abstraction is reduced to a more reasonable value of 88.4 kcal/mol. Clearly there are also other possibilities. Another hydrogen bonding second sphere ligand could be present, or there may be shifts between five- and six-coordination during these abstractions. Model calculations of these situations are in progress, but for a complex system like PSII, where there is only a fragmentary knowledge of the actual ligands, most problems cannot be expected to be entirely solved at this stage. Nonetheless, the calculations presented here have provided considerable insight into feasible reaction mechanisms in the oxygen evolution process.

Conclusions

There are several general and some more specific conclusions that can be reached from the work presented here. It has been found that coordination of a water molecule to a manganese ion substantially lowers the energy needed to abstract one or two hydrogen atom(s) from the water molecule. Specifically for PSII, the decrease in bond dissociation energy is enough to make hydrogen abstraction from water possible for the nearby tyrosyl radical, Tyr_Z. Furthermore, such a hydrogen transfer would proceed almost without energy losses. Thus, these results provide a sound theoretical basis for the recently proposed^{19,20,23} mechanism for the oxidation of water to molecular oxygen in which oxidized Tyr_Z directly abstracts hydrogen atoms from water in a series of reactions, ultimately leading to the formation of molecular oxygen from two oxidized water molecules. These conclusions from our computational approach are generally consistent with recent thermodynamic cycle calculations on the permanganate ion by Gardner and Mayer.³⁸ Even more interesting is the agreement between the present results and the

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experimental results for various OEC model complexes performed by Pecoraro et al. during the same period as the present calculations were in progress.⁴³ In addition, our results show that it might become possible to predict, by these kinds of calculations, the functional role of important cofactors like chloride or calcium and to make contributions toward our understanding of how the last step, the formation of molecular oxygen, takes place in PSII.

Our results may also be applicable in a more general sense. Long-range electron transfer is important in many biological reactions including not only photosynthetic systems but also enzymes important in DNA metabolism (for example, ribonucleotide reductase), in respiration (cytochrome oxidase and other enzymes), and in detoxification. In some of these systems, chains of amino acid side chains (sometimes even identified⁴⁴) are thought to constitute electron transfer pathways between

metal and/or radical centers. It is not unlikely that hydrogen transfer will prove to be a universal phenomenon and that what is now thought to involve long-range movement of electrons (electron transfer (ET)) sometimes may be better described as hydrogen atom transfer. We have recently studied different hydrogen transfer mechanisms in the presence of amino acid radicals in a general context by using selected model systems.⁴⁵

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