Manganese Oxyl Radical Intermediates and O−O Bond Formation in Photosynthetic Oxygen Evolution and a Proposed Role for the Calcium Cofactor in Photosystem II

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Abstract: Spin state considerations are proposed to sharply limit the possible O−O bond-forming steps in water oxidation by the oxygen evolving center of Photosystem II. A series of intermediates are proposed for the Kok S states on the basis of quantum chemical studies on simple model complexes; these are also consistent with the main biophysical data. Only one Mn atom in the active site cluster is thought to be redox-active and mediate O−O bond formation. A key concept is the formation of an unreactive Mn=O oxo at the S_2 state, followed by its conversion to a reactive Mn−O• oxyl form at the S_3 level, with radical character on the oxyl oxygen, at which point O−O bond formation can occur by a coupling between the oxyl and an outer-sphere water molecule. An MnOOH intermediate at S_3 is proposed to lose a hydrogen atom to give O_2. The role of the Ca cofactor is to bring about a 5- to 6-coordination change at S_2, necessary for formation of a reactive oxo in S_3. The chloride cofactor is assigned the role of charge neutralization.

I. Introduction

Despite intense interest in the mechanism of dioxygen evolution by the oxygen evolving center (OEC) of Photosystem II (PSII) in green plants, the precise mechanistic steps involved and, in the absence of a crystal structure, even the structure of the OEC itself remain matters of current debate.[1] One calcium and four manganese ions per PSII complex are all essential for oxygen evolution, and one chloride cofactor is also present but its removal does not completely suppress O_2 evolution.[2] Mainly on the basis of EPR and EXAFS studies, particularly the presence of an EXAFS peak assigned to a Mn···Mn separation of 2.7 Å,[1a] the manganese ions are generally believed to be arranged in two pairs of dinuclear manganese clusters each containing a Mn(μ-O)$_2$Mn core, a very common structural motif in inorganic model compounds.[1f−h] The two Mn dimers, the Cl, and the Ca are believed to be close to and probably even linked together in the OEC. As a redox-active metal, attention has naturally focused on the role of the manganese, where the substrate water is believed to bind and undergo oxidation. The roles of chloride and calcium are much less well defined,[1d] chloride usually being considered as a probable ligand for Mn or Ca or both.

In photosynthetic oxygen evolution, each photon absorption event leads to an electron transfer which oxidizes the P680 chromophore of PSII to the P680•$^+$ cation radical. This radical

in turn oxidizes the OEC by one electron via the intermediate oxidation of a tyrosine residue, denoted Y$_z$. From the results of a saturating flash experiment, which showed maximal O_2 evolution on the 3rd, 7th, and 11th flashes, Kok[3] proposed a generally accepted scheme in which the OEC accumulates oxidizing equivalents by advancing it in one-electron (1e) steps from an S_0 oxidation level through S_1, S_2, and S_3 until at the most oxidized S_4 oxidation level, O_2 is released, and the cluster returns to S_0. There is an indication from EPR spectroscopy that alternative states of the cluster are possible at the S_2 state because two different EPR signals (the multiline signal at $g = 2$ and the $g = 4.1$ signal) are seen,[4] depending on the temperature and sample preparation.[2] The invariance of the EXAFS Mn K-edge on going from S_2 to S_3 suggests that the Mn atoms are not oxidized in this step;[5a] contrary conclusions have been reported, however.[5b]

Messinger and co-workers[6] have shown by H$_2$^{18}O labeling that, of the two O atoms in S_2 and S_3 that become O_2 after S_4, one is in a slowly exchanging site and the other in a very rapidly exchanging site. The slow exchange site has been considered to be a terminal oxo, and the fast exchange site, a water or hydroxo group. In any case a symmetrical Mn=O + O=Mn mechanism seems to be unlikely.

Ca depletion abolishes O_2 evolution, but reconstitution with Sr partially restores activity and modifies the EPR spectrum of

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the "multiline" EPR signal that is known to arise from the S_2 state of the Mn cluster, suggesting that Ca may be close to the Mn cluster. Indeed Mn EXAFS data have been interpreted by MacLachan[8a] and by Yachandra, Sauer, Klein, and co-workers[8b] in terms of a 3.7-Å Ca···Mn separation, although other interpretations exist.[9] Ca-depleted samples are inactive because $S_2Y_z\bullet$ is the highest state that can be attained. Replacement of chloride by bromide[10] does not affect activity, but iodide partially and fluoride completely inhibits O_2 evolution. Fluoride binding appears to elongate the 2.7-Å EXAFS Mn···Mn vector to 2.8 Å, suggesting binding at or near the Mn cluster.

Important information for making Mn oxidation state assignments for the S states comes from NMR proton relaxation studies (NMR-PRE),[11a] where Mn(II) and Mn(IV) are strongly relaxing and Mn(III) weakly relaxing ions. Microwave power saturation studies[11b] of the SII_s EPR signal of PSII can also probe the oxidation states involved. In conjunction with XANES data,[5] both NMR and EPR results suggest that a Mn(II) exists in S_0, that one Mn(III) is oxidized to Mn(IV) in the S_1 to S_2 conversion, and that no change in Mn oxidation state occurs on going from S_2 to S_3. An extra paramagnet produced in S_3 and coupled to the Mn cluster is thought[11b] to prevent observation of the S_2 MLS in S_3.

Babcock et al.[12a,b] have proposed H-atom abstraction from bound water as the key step in OH bond breaking and the role of metalloradicals in O_2 formation via a Mn(IV)=O/(HO)Mn-(IV) coupling induced by H transfer to $Y_z\bullet$. Pecoraro et al.[12c] have experimental evidence from model compounds that μ−O−H bonds are relatively weak in Mn_2(III,IV) dimers, consistent with H abstraction being important. Yachandra, Sauer, and Klein have considered a pair of bridging oxyl radicals as precursors to O−O bond formation.[1a] Both Pecoraro[12d] and Brudvig[7b] have discussed O−O bond formation via nucleophilic attack by a hydroxo group on a Mn(V)=O oxo species. The proposed overall requirement for charge neutrality has also been emphasized by several authors as most appropriate for a cluster buried deep in the low dielectric medium of the protein.[12a,b,13]

Krishtalik[14] has proposed that O−O bond formation proceeds via concerted multielectron redox steps, either $2 \times 2e$ or $4e$, rather than via four 1e steps, even though the OEC is indeed oxidized in 1e steps. To avoid excessive charge separation, this implies strong coupling between proton- and electron-transfer events.[12,13]

This paper develops the quantum chemical work reported earlier[13,15] on the photosynthetic O_2 evolution problem. The prior work examined pathways by which the O−H bonds of water might be broken[13] and how the O−O bond might be formed[15] in the OEC, and in this paper, we look at the properties of the resulting oxo complexes and at possible roles for the Ca cofactor in promoting O−O bond formation. In addition, a number of general principles emerge which strongly constrain possible pathways. This work helps exclude what might otherwise be considered reasonable mechanisms but permits a number of possibilities, which we discuss.

II. Computational Details

The calculations follow the pattern set in the prior paper,[13] with a geometry optimization performed using B3LYP,[16] a density functional (DFT) calculation based on hybrid functionals and double-ζ basis sets (LANL2DZ). In a second step, the energies were evaluated at the optimal geometry using basis sets with polarization functions (LANL2DZ + polarization and diffuse functions from 6-311+G(1d,1p)). The final energy evaluation was also performed at the B3LYP level. All calculations were carried out with the GAUSSIAN-94 set of programs.[17] Other details were exactly as previously described.[13]

Reaction barriers were of great interest, especially for the O−O bond-forming step. In this first report on the new mechanism for O−O bond formation, a simplified procedure was adopted for locating approximate transition states allowing several different pathways to be investigated efficiently. A good approximation for a transition state can usually be obtained by constraining a critical interatomic distance to change stepwise over the ts region and optimizing at each step. In the present case where an O−O bond is formed, constrained O−O distances of 2.0, 1.9, and 1.8 Å provided a good indication of the viability or otherwise of a given transformation. It was sometimes necessary to vary another bond distance, such as an O−H, to search most effectively for the lowest barrier pathway. It is expected that the error on the estimated barrier height using this procedure should be at most a few kilocalories per mole, which is sufficient for the present purpose. When O−H bond strengths are discussed below these were obtained in the following way. To the calculated value using the largest basis set was subtracted a zero-point effect of 6.2 kcal/mol, based on the effect obtained at the B3LYP level for a water ligand in a 5-coordinate Mn-(IV) complex. A systematic basis set and method correction to the O−H bond strength of +1.7 kcal/mol was also added to the values discussed below, based on the error obtained for a free water molecule. The O−H bond strength of tyrosine is taken to be the same as in phenol, 86.5 kcal/mol. The dielectric effects from the surrounding protein were obtained for a few model systems using the self-consistent isodensity polarized continuum model (SCI-PCM) as implemented in the GAUSSIAN-94 program.[18] The dielectric constant was given a value of 4. Zero-point vibrational effects, which were found to be very small for the present reactions, were also obtained only for a few model systems at the B3LYP level.

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III. Results and Discussion

A key point of this work is that the final mechanistic proposal presented was reached on the basis of a few quite general results and principles that appear to rule out a very large number of otherwise viable pathways. While the proposed pathway is the most satisfactory one so far found, others may well be possible. Although improvements will no doubt be discovered in future, we believe that the true mechanism is likely to conform to the general results and principles and contain many key elements of the present proposal. We therefore emphasize these points in our discussion.

In choosing suitable quantum chemical models, we have adopted the same simplifications as in prior work.[13] For computational simplicity, H_2O and OH ligands are chosen to model the more complex neutral and anionic ligands, respectively, provided by the protein. We choose to use complexes or clusters that carry no net ionic charge both for computational simplicity and on the proposed grounds that overall neutral clusters are most likely to be present in the real system because of the low dielectric constant of the protein medium. As in the prior work, a pair of *cis*-OH groups have been used to model the proposed μ-oxo groups of the real system, a simplification which allows much more efficient testing of the many hundreds of structures that needed to be examined. The calculations reported here have also been carried out on a mononuclear manganese site. This not only is a computational simplification but also may be most relevant to the OEC, where, for reasons discussed below, one Mn ion (denoted Mn_A) may undergo all the redox changes, as others have also suggested previously on the basis of biophysical data.[1a] Of course, at all stages, comparisons with the available biophysical data have also guided us in the choice of models to test.

The energies of quantum chemical models for proposed S-state intermediates provided a very severe test of their viability within the assumptions of the model. An intermediate or transition state with an energy too low or too high relative to the prior or subsequent intermediate was eliminated as irrelevant. For example, a mechanism leading to an O–O formation barrier higher than 20 kcal/mol or one requiring hydrogen abstraction of an O–H bond stronger by more than 10 kcal/mol compared to the O–H bond of tyrosine was dismissed. Even assuming negligible intrinsic reaction barriers, any endothermicity in any of the steps will result in a barrier for that step. Considering that the overall exothermicity of the PSII water oxidation for the entire reaction pathway is rather modest (14.0 kcal/mol, calcd[13]), only a series of intermediate structures having energies in a relatively narrow descending range can reasonably be considered. Any intermediate that is too stable would lead to an unreasonably high barrier for the next step, and any intermediate with too high an energy could not reasonably be reached via a low barrier from the previous S state. Having established the energies of possible intermediates, the next test is whether low-barrier transformations can be found for the key step. In future studies we hope to test the barriers for all of the proposed steps.

We cannot test all possible models, of course, so this report can only be considered as a first step in the process of better defining the mechanism of water oxidation by the OEC. We hope that the testable predictions that do emerge here will stimulate future experimental work, in both the biophysical and inorganic areas.

Quantum chemical results obtained previously[13,15] suggest that, with the present type of weak-field ligands, Mn(IV) has a much stronger preference for 6-coordination than do Mn(II) and Mn(III), where 5-coordination is also accessible; for Mn(V) oxo species, 5-coordination is preferred with a vacancy trans to the oxo group. The ground states are always high-spin.

a. The Potential Surface Problem. The first key general issue addressed here is the nature of the potential surface on which the O–O bond formation can occur. For the present weak-field manganese complexes, the spin is always the highest possible for a given oxidation state, so there is a one-to-one correspondence between a given potential surface and the spin state of the local manganese atom. The spin coupling between different manganese atoms is usually rather weak: it was shown in a previous theoretical paper[13] that the ferromagnetic and antiferromagnetic spin coupled forms of a typical manganese μ-oxo dimer differ in energy by only 2 kcal/mol and that the geometric structures for these two forms are virtually identical. Even if this energy difference were as high as 4 kcal/mol,[19a] as suggested by a reviewer, it would still be very unlikely to have a major impact on the mechanism since the energy effect is not a direct but a second-order effect. For a quantitative rate determination of O–O bond formation the spin coupling issue may of course still be non-negligible, but this goes beyond both the purpose of the present study and the accuracy of the quantum chemical methods used. We therefore propose that the present arguments do not change whether the metal complex is composed of one or many manganese atoms. In the following we therefore describe a potential surface by its local manganese spin. The spin-state (or potential surface) issue is a fundamental problem in dealing with redox reactions involving paramagnetic complexes or clusters as is commonly the case in bioinorganic chemistry. This issue has not gained wide attention, but it is expected to be important and may often prove to have decisive consequences for the mechanism.

In the simplest case of a chemical reaction, the same PE surface links starting materials and products. More typically for redox reactions in a paramagnetic metal cluster, the starting and final species are on different potential surfaces and therefore have different ground spin states. In such a case, there must be a transfer from the starting surface to the product surface at some point as we go from starting materials to products. In the case of the OEC Mn cluster, a reduction of the Mn ions occurs as the O–O bond is formed in one of the later S states, which for the present ligands means that the local spin on manganese increases in this step. This leads directly to a spin-state problem of the type mentioned. To take a specific example, if a 5-coordinate complex $(H_2O)(HO)_3Mn(V)=O$ were to convert to $(H_2O)(HO)_2Mn(III)-O-O-H$ in one step of O_2 formation, the spin would rise from triplet in the starting material to quintet in the product; similar changes happen whatever the precise structures involved.

In the O–O formation step there are two different possibilities. The reaction could occur on the potential surface of the reactants or on that of the products. If the reaction occurs on the ground-state potential surface for the reactants, an excited state of the products will be formed. If, on the other hand, the reaction occurs on the ground-state potential surface for the products, the reaction has to start in an excited state for the reactants. There is in practice no third alternative as has been discussed, for example, for the simple reaction between a Ni atom and water[19b] and also by Shaik and Schwartz et al.,[19c] who recently considered this point in the case of Cyt P-450.

The two possibilities above have been investigated for a

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5-coordinate Mn(V) oxo species, $(H_2O)(HO)_3Mn(V)O$ (**1**), that plays a key role in our discussion. As an O−O bond is formed between the oxo ligand and an OH ligand, a 5-coordinate Mn(III) peroxo species, $(H_2O)_2(HO)_2Mn(III)(O−OH)$ (**2**), is formed,
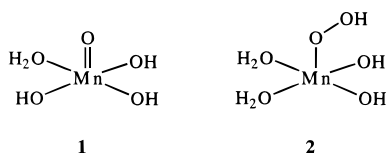
by coordination of an external water molecule. The ground states and the first excited state of both reactants and products were optimized, with the results anticipated above. For the reactant oxo complex the lowest excited state is best considered as a manganese oxyl of type Mn(IV)−O• because it has a very high unpaired spin density (ca. 0.9) on the oxo oxygen, which therefore has oxyl character. The excitation energy from the ground state is 26 kcal/mol. This excited state is ideally suited to form the O−O bond since it has already broken the Mn=O π bond and a radical is expected to be particularly reactive. The excited state of the peroxide product has, as expected, just two of the spins on manganese paired up compared to the high-spin ground state. Apart from this change both the wave function and the structure are almost identical in both spin states. There is no sign of any incipient Mn=O formation or breaking of the peroxide O−O bond. Even though the excitation energy is almost as low as for the reactant, 29 kcal/mol compared to 26 kcal/mol for the product, the reactant excited state appears to be the most appropriate surface for the O−O bond-formation reaction; this choice will be assumed for the rest of the paper. An exactly similar reasoning can be applied for a case where the O−O bond is formed between oxygens on two different manganese atoms, such as between an Mn(V)=O and an Mn(IV)−OH forming an Mn(IV)−O−OH and a Mn(III) product. Again a change of potential surface is required, and again the reactant excited state with Mn(IV)−O• character is ideally suited for O−O bond formation, while the product excited state has little relation to O−O bond formation. These results are fairly straightforward and hardly need to be demonstrated by explicit calculations, but nevertheless, they constitute the fundamental building block on which the present mechanism for O−O bond formation has been constructed.

Figure 1 shows in schematic form the situation that applies to the 5-coordinate Mn(V) oxo species, $(H_2O)(HO)_3Mn(V)O$ (**1**). The ground-state spin triplet Mn=O state has a typical double-bonded $d(MnO)$ distance of 1.6 Å and an energy 26 kcal/mol below that of the lowest point on the high-spin quintet surface, which has a single-bonded $d(MnO)$ of 1.8 Å. The high-spin form is, as mentioned above, best considered as an oxyl of type Mn(IV)−O• because it has a very high unpaired spin density (ca. 0.9) on the oxyl oxygen. Each point in the figure is obtained by a full geometry optimization of all degrees of freedom except $d(MnO)$.

In any reaction of the Mn=O ground-state spin form of **1**, say the conversion of HO−Mn(V)=O to Mn(III)−O−O−H, we expect the Mn=O bond to elongate from a double bond in the reagent to a single-bond distance in the product. The energy is found to rise strongly, being only 11 kcal/mol below that of the high-spin state at the preferred $d(MnO)$ for the high-spin state. This is reasonable because at this long $d(MnO)$ pairing the spins in the Mn−O• form to make the additional bond in Mn=O is expected to lead only to a very weak MnO π bond and therefore only to a rather small energy decrease. The main reason the triplet state is below the quintet state at 1.8 Å is
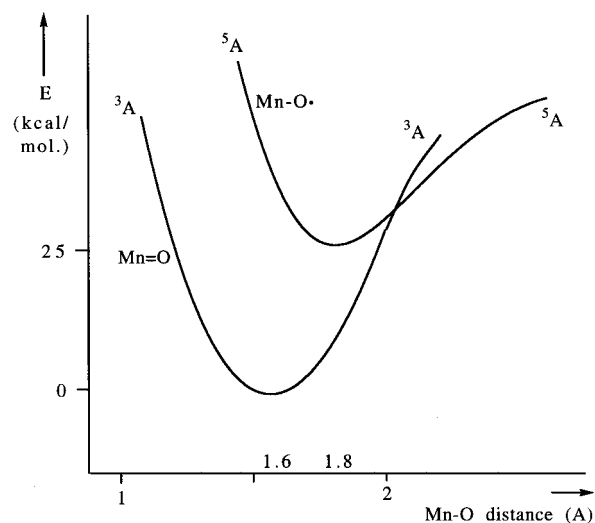

Figure 1. Schematic diagram showing the relative energies of the ground-state ^3A Mn(V)=O and excited-state ^5A Mn(IV)−O• forms of the 5-coordinate and formally Mn(V) oxo species, $(H_2O)(HO)_3MnO$ (**1**).

that, while the quintet has a fully broken π bond, the triplet retains some π-bonding and instead prefers to have some part of the σ bond broken. When a transition from one spin to the other is needed, it is expected to occur close to the high-spin-state minimum, where the curvature of the potential surface is smallest and where the structural reorganization and energy change would both be minimal. Stable, 5-coordinate Mn(V)=O compounds are singlets, so the spin change would be even larger and the reaction even less favorable if such a species were invoked.

From the above it is clear that O−O bond formation would be much easier if the excited Mn−O• oxyl state were easily reached. This is not the case for the 5-coordinate model complex **1** discussed above, which requires a prohibitively high 26 kcal/mol excitation energy to reach the Mn−O• state. The problem is thus to find a situation where this excitation energy is much lower, preferably even zero.

Unfortunately, even though the high-spin-state Mn−O• species can be highly reactive, it is not sufficiently reactive for O−O bond formation in many situations. For example, simply bringing the oxyl and a *cis*-hydroxyl ligand together in **1** to form an MnO−OH ligand on the oxyl potential surface leads to a substantial barrier of more than 30 kcal/mol. This means that the total barrier for O−O formation for this complex is higher than 55 kcal/mol, which is the sum of the excitation energy and the subsequent barrier on the excited-state surface. The high barrier is presumably the result of the difficulty of deforming the Mn octahedron, but even if an additional outer-sphere water molecule is added to the structure as discussed below to allow O−O bond formation with minimal deformation, the barrier on the oxyl surface is still a high 25 kcal/mol, leading to a total energy cost of 51 kcal/mol. To arrive at a reasonable total barrier for O−O bond formation therefore requires us to find not only a structure where the oxyl state is very low in energy but also a pathway on this surface which has a low barrier for O−O bond formation.

b. Structures with Low-Lying Oxyl States. The first problem to be solved to develop a mechanism for O−O bond formation is thus to find the structural requirements which make the oxyl state easy to reach. Early investigations quite clearly revealed one situation which stabilizes an oxyl state relative to

the oxo state.[15,20] In a systematic investigation of manganese complexes with different oxidation states and coordination, it was found that 6-coordinate, formally Mn(V)=O oxo complexes with the present types of ligands, do indeed have Mn(IV)−O• oxyl ground states. This perhaps quite surprising result is the most important prior information forming the basis for the present mechanism. Several factors favor going to the octahedral Mn(IV)−O• oxyl state: the large trans effect of the oxo group, the high cost of staying in the high Mn(V) oxidation state, and the strong preference of d^3 Mn(IV) for an octahedral geometry. The importance of the high oxidation state is best seen by comparison to the analogous Mn(IV)=O complexes, which are expected to have the same trans effect but which still have Mn-(IV) oxo and not Mn(III) oxyl ground states.[20]

Highly reactive ground states do indeed seem to be possible for 6-coordinate manganese oxo complexes as shown by recent experiments[21] on a model Mn(III,IV) system [LXMn(μ−O)$_2$MnLY]$^+$ (L = terpyridyl; X = Y = anion) that evolves oxygen on exposure to oxone (peroxysulfate) and other O-atom donor reagents. According to recent quantum chemical results,[21] the ground state of the corresponding 6-coordinate Mn(IV,V) oxo, [LXMn(μ−O)$_2$MnOL]$^{2+}$ (**3**), assumed to be formed after

3

(py = terpyridyl binding site)
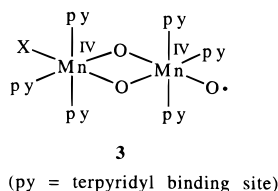
O-atom transfer, is indeed expected to have a Mn(IV)−O• structure and to permit facile O−O bond formation, either via reaction of two Mn(IV)−O• groups or of one Mn(IV)−O• with a water molecule from solution.

6-Coordination is not the only situation where an oxyl state becomes low-lying. In a theoretical investigation[15] of several hundred different manganese dimer structures to look for possible oxyl character, the most interesting one found was a 5-coordinate Mn(III,V) dimer, **4**. In this structure a strong Jahn−

4
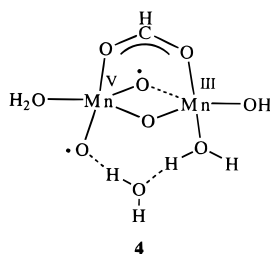
Teller (JT) distortion of the Mn(III) center helps to introduce large radical character both on the oxo ligand and on one of the μ-oxo groups. The oxyl excited state was found to be only 8 kcal/mol above the oxo ground state for **4**. However, further studies of **4** showed that the MnO−H bond strength of plausible precursors was always far too strong for abstraction by a tyrosyl radical as required in the OEC. Finding no way to fit **4** into a plausible catalytic cycle for the OEC, we went on to look for a general pattern: was high oxyl character always accompanied by high MnO−H bond strength in the natural precursor?

(20) Blomberg, M. R. A.; Siegbahn, P. E. M. *Mol. Phys.*, in press.
(21) Limburg, J.; Brudvig, G. W.; Crabtree, R. H. *J. Am. Chem. Soc.* **1997**, 119, 2761. Limburg, J.; Brudvig, G. W.; Crabtree, R. H.; Siegbahn, P. E. M. **1998**, unpublished data.

c. Formation of an Oxyl Structure: a Dilemma. From the results and the discussion above, there are two requirements for the O−O bond-formation step. We need first to find a structure with a low-lying oxyl state and then to find an efficient O−O bond-formation pathway for it on the oxyl surface. It would seem that the first problem is nearly solved: we merely need to form a 6-coordinate complex where the high-spin form is the ground state. Unfortunately, a major problem now appears that prevented significant progress for a year. In each step of the O$_2$-formation cycle, a tyrosyl radical is produced by the energy obtained from the photon absorption, and at the end of each step, a tyrosine is recreated. As proposed by Babcock and co-workers,[12a,b] found experimentally in models by Pecoraro and co-workers,[12d] and supported in a prior quantum chemical paper,[13] this is likely to occur by H-atom abstraction (proton-coupled electron transfer) from water or hydroxyl ligands bound to the manganese complex. Considering the low dielectric medium of a protein, this process appears reasonable in light of the proposed preference for neutrality of the manganese cluster and the avoidance of energetically expensive charge separation in the transfer of a neutral H atom versus transfer of a proton and an electron separately. Even if the true process were a decoupled electron transfer and proton transfer, we would still have net H-atom transfer and so the energy relationships in the system would still be well represented.

The prior quantum chemical studies[13] indicate that H-atom abstractions by tyrosyl from water or hydroxyl ligands coordinated to low-valent manganese do indeed give close to thermoneutral processes. The O−H bond strengths for a variety of water and hydroxo complexes of Mn(III) and 5-coordinate Mn-(IV) were generally found to lie in a ±3 kcal/mol range relative to the corresponding value for TyrO−H (86.5 kcal/mol). Near thermoneutrality is a requirement for the enzyme because the overall process of eq 1 is only weakly exothermic (14 kcal/

$$4TyrO• + 2H_2O = 4TyrO−H + O_2 \qquad (1)$$

mol, calcd) and there can be no excessive energy drop in any one step. The requirement that each H abstraction be slightly exothermic places rather severe restriction on the types of water complex that can be successfully used. If the O−H bond strength is only slightly increased from the values noted above, the overall reaction would cease to be feasible. To put it another way, given the OH bond strength of TyrO−H, only certain types of oxo complexes can be directly obtained via H abstraction from Mn−OH by the route of eqs 2 and 3.

$$Mn(II)(HO−H) + TyrO• = Mn(III)O−H + TyrO−H \qquad (2)$$

$$Mn(III)O−H + TyrO• = Mn(IV)=O + TyrO−H \qquad (3)$$

Equations 2 and 3 provide us with a straightforward way to make oxo ligands of Mn(IV) or Mn(V) depending on the oxidation state of the precursor hydroxy complex, but as we have seen, a further requirement is that the oxo complex formed at S$_3$ or S$_4$ be a reactive oxyl complex, capable of forming O−O bonds.

A key result of quantum chemical work on H abstraction from Mn(IV)−OH precursors to give formally Mn(V)O oxo species is that, if the MnO−H bond strength is in the range that allows slightly exothermic H abstraction by the TyrO• radical, the Mn=O state is so much more stable than the reactive Mn−O• state in the product oxo that subsequent O−O bond formation has a barrier that is far too high.[15] Conversely, a reactive oxo cannot be formed from a MnO−H precursor because the O−H bond strengths are now too high. The strengths of O−H bonds leading

to oxyl complexes after hydrogen abstraction are typically 20 kcal/mol larger than that in tyrosine. No other H-abstraction pathways leading to the same 6-coordinated oxyl complex are possible either. For example, starting from a 6-coordinate (H_2O)-Mn(III)(OH) complex and first going to a (H_2O)Mn(IV)=O complex and then to the final (OH)Mn(IV)O• complex is not a solution, since the same excessive energy difference between the starting Mn(III) and the final Mn(IV)O• complex must be independent of intermediate states. In this case both the first and the second hydrogen abstractions are about 10 kcal/mol endothermic. In going from the (H_2O)Mn(III)(OH) to the (OH)-Mn(IV)(OH) and then to the final Mn(IV)O• complex, the first step is thermoneutral while the second step is endothermic by 20 kcal/mol, giving the same energy difference between the Mn-(III) reactants and the final products for the two pathways, as required by Hess's Law. So far we have not found any exception to the result that pure H-abstraction steps cannot lead to the desired oxyl state. We are therefore left with an apparent dilemma: reactive oxos cannot realistically be formed, and only unreactive oxos are accessible under the conditions that prevail in the OEC of PSII. These results do not change the general conclusion that the oxyl state potential surface is the platform necessary to initiate O−O bond formation. What is not clear at this stage is how to reach this state. Our proposal of an oxygen-centered radical therefore seems to be quite different from that in prior work,[12b] in which it was suggested that oxidation to produce an oxygen-centered radical could be ruled out.

d. A Role for Calcium. We propose that the role of the calcium cofactor in PSII is to provide sufficient free energy from chelation to the Mn cluster to allow the formation of the 6-coordinate Mn(IV)O• oxyl state. The nature of the 5-coordinated precursor state cannot be completely specified because the "5-coordinate" form could easily have a long bond to a sixth ligand; in such a case, Ca might be "weakly chelated" to the Mn cluster. The process may occur as follows. A 5-coordinate (or weakly chelated $(5 + 1)$-coordinate) oxo complex is first created in the Mn dimer without, or with only weak chelation, by the Ca complex. This should be possible by a straightforward H abstraction by the tyrosyl radical from a terminal Mn−OH on the dimer, as demonstrated in earlier quantum chemical calculations.[13] As a "5-coordinate" species, this Mn-dimer oxo complex should be entirely unreactive and is not expected to be capable of forming an O−O bond at this stage. After this step is entirely completed, the Ca complex is expected to engage in strong chelation to the Mn dimer, and in this process, Mn_A, the manganese closest to the Ca ion, will go from 5-coordination to 6-coordination. This in turn should force the creation of the oxyl radical in this step or in a later one, depending on the oxidation state of the manganese. The reason we propose this chelation process does not occur until the stable oxo complex has already been formed is that until that stage manganese is either Mn(II) or Mn(III), which does not strongly prefer 6-coordination. Since the oxo should only be formed when the oxidation state is raised to Mn(IV), 6-coordination becomes much more favorable, as mentioned above.

In the S-cycle scheme, there seem to be two possibilities for incorporating chelation by Ca, as will be discussed here and in the next subsection. The first is that an initially formed "5-coordinate" Mn(V)=O species will chelate with the Ca complex and in this process become 6-coordinate, forcing the complex to adopt an Mn(IV)−O• oxyl state. The second is that chelation takes place to convert a "5-coordinate" (H_2O)Mn(IV)=O complex to the 6-coordinate analogue. No ground oxyl state should be obtained at this stage because the oxidation state is

4+. Only in the next step, when a hydrogen is abstracted from a adjacent water molecule that bridges Mn and Ca, can the desired Mn(IV)−O• oxyl complex form. The second H-abstraction step, which is not possible for an unbridged manganese complex (see above), is aided by the increase in binding energy in going from a chelating water to a chelating hydroxyl group. Calcium complexes are perfectly suited for this type of chelation since they provide strongly anionic ligands giving optimal energy gains in these processes. It is the second of these possibilities that we propose is most likely to occur in the enzyme, since in this way the tight chelation occurs the first time manganese reaches oxidation state 4+, where 6-coordination is relatively favored. If the chelation occurs for Mn(V)=O, it is more difficult to understand why this did not happen one step earlier for Mn(IV)−OH, but chelation cannot occur for Mn(IV)−OH since it would then become impossible to remove the hydrogen from the Mn−OH group because 6-coordination leads to an excessive O−H bond strength.

Finally, there is a potential problem with the above scheme. As the Mn(IV)−O• state is formed, the oxyl ligand can abstract hydrogen atoms with X−H bond strengths as much as 20 kcal/mol greater than in tyrosine. This is not enough to abstract hydrogens from free water or from water bound to the Ca complex, but the active tyrosine Tyr_Z-OH and perhaps $Mn_2(\mu\text{-}OH)_2$ groups are also likely to be in the neighborhood. This is a dangerous situation for O−O bond formation because if a hydrogen atom is abstracted by the oxyl ligand we then risk immediately quenching the reactive species we have just made, thus arresting the catalytic cycle. We fully accept this difficulty with the proposal, but we do not regard it as fatal because we believe the protein could adopt a conformation that prevents parasitic hydrogen transfers. And, from the several hundred structural investigations so far made, we do not see any alternative that avoids this situation.

e. A Proposed Sequence of S-States. With these ideas in hand, we are left with little or no choice in proposing structures for the S states, which are shown in Figure 2. The major constraint on this choice is that the reactive 6-coordinate Mn-(IV)−O• state has to be obtained by H abstractions from two different water-derived ligands on the same manganese center. This follows from the fact that hydrogen atoms cannot be abstracted from a 6-coordinate Mn(IV)−OH group, an abstraction shown by numerous model calculations[13] to be endothermic by about 20 kcal/mol; no exceptions have been found so far. Instead, the precursor for the Mn(IV)−O• state has to be a 6-coordinate (H_2O)−Mn(IV)=O species, from which the reactive oxyl state is obtained by hydrogen abstraction from the water ligand. This means that both water molecules involved in the redox chemistry have to be bound to the same manganese atom in the precursor to the oxyl state, essentially excluding mechanisms where waters on different manganese centers are involved. Furthermore, the Ca complex has to take an active part in this process, as discussed in subsection IIId.

The active manganese (Mn_A) in S_0 should contain an (H_2O)−Mn(II)−(H_2O) group. The first hydrogen abstraction should then lead to an (H_2O)−Mn(III)−OH for Mn_A in the S_1 state. The second H abstraction then leads to (H_2O)−Mn(IV)=O in the S_2 state, which makes a suitable precursor for the reactive oxyl state. In these two steps, particularly in the first one, an alternative site for H abstraction is a bridging $Mn_2(\mu\text{-}OH)$ group; this causes no essential change in the mechanism. Model calculations and experimental experience both suggest that water coordinated to Mn(II) has a tendency to lose a proton to a bridging μ-oxo group. These protons will then be equally well abstracted by the tyrosyl radical. Model calculations[13] also show

that both Mn(II) and Mn(III) tolerate 5-coordination with the present type of weak-field ligands, so up to this point, the active manganese is not expected to be 6-coordinate.

The assignments of the S_0, S_1, and S_2 states are consistent with the main experimental information available, as confirmed by the similarity of the oxidation states we suggest with models previously proposed by Klein et al.[1,5] and by Babcock et al.,[12b] who both relied purely on experimental information. In particular, the NMR-PRE,[11a] EPR power saturation,[11b] and the XANES[5] data indicate Mn cluster oxidation state changes with S state that are entirely in accord with all three proposals.

The 5-coordinate S_2 state described above as $(H_2O)-Mn-(IV)=O$, and possibly identical with the $g = 4.1$ state observed by EPR, may chelate to Mn_A and cause the reorganization from 5- to 6-coordination that is necessary for attainment of the proper S_3 state. An example of such a rearrangement is shown as $S'_2 \rightarrow S''_2$ in Figure 2. This 6-coordinate species could give rise to the multiline signal observed by EPR. A rearrangement of the S_2 state has been noted experimentally, for example, by local electrochromism and Bohr effects that Tommos and Babcock[12a] interpreted as resulting from an internal charge rearrangement, consistent with our model where the $CaClL_n$ complex is involved in the rearrangement.

The next step forms the S_3 state, assigned in our model as the reactive $(OH)-Mn(IV)-O\bullet$ oxyl. This state is shown by the present model calculations to be nearly isoenergetic with a few other isomers. One is a pure 5-coordinate $Mn(V)=O$ oxo state with loose binding to the Ca complex (see below). In another, the reactive oxyl species rearranges to form an $Mn-(III)-O_2H$ complex, also discussed below. According to our model calculations, three different oxidation states should thus be very close in energy. The present suggestion of $(OH)-Mn-(IV)-O\bullet$ oxyl state for the real S_3 is based on the experimental NMR-PRE,[11a] EPR,[11b] and some[5a] of the XAS observations on S_3 that suggest that no Mn oxidation or reduction takes place on going from S_2 to S_3. Solely on the basis of the present calculations, no definite assignment can be made, however, since the three alternatives are all too close in energy. The structure adopted by the real S_3 state may therefore be a consequence of a fine-tuning of the energies as discussed in subsection IIIg and is not a critical part of the present mechanism.

At S_3, the reactive $(OH)-Mn(IV)-O\bullet$ oxyl is considered to be in thermodynamic equilibrium with a $Mn(III)-O_2H$ complex, where an $O-O$ bond has been formed; this equilibrium is expected to slightly favor the oxyl. In the fourth and final H-abstraction step, the tyrosyl radical can abstract a hydrogen from the MnO_2H group, leading to O_2 release as the transient S_4 state rapidly reverts to S_0. The $O-O$ bond formation to give MnO_2H has the highest barrier and is rate-limiting. Since it occurs before the much faster H abstraction from MnO_2H, this explains the observation[12a,b] that $Y_z\bullet$ reduction and O_2 release occur with the same rates.

The model is also in accord with the Messinger ^{18}O-labeling studies[6] because one O of O_2 comes from the slow-exchanging oxo and the other from the fast-exchanging second-coordination-sphere water. The model provides a natural explanation for the OEC not being able to proceed past $S_2(Y_z\bullet)$ when Ca is absent, because without the Ca, the Mn cannot become fully 6-coordinate and therefore generate the reactive oxyl group. The Krishtalik[14] ideas are also obeyed, the $O-O$ bond-formation pathway being of the $2 \times 2e$ type. The MacLachan estimate of the $Mn\cdots Ca$ distance in S_1, 3.7 Å, is easy to reconcile with the present model because the Ca is not expected to be doubly bridging at this point in the cycle and so would relax somewhat
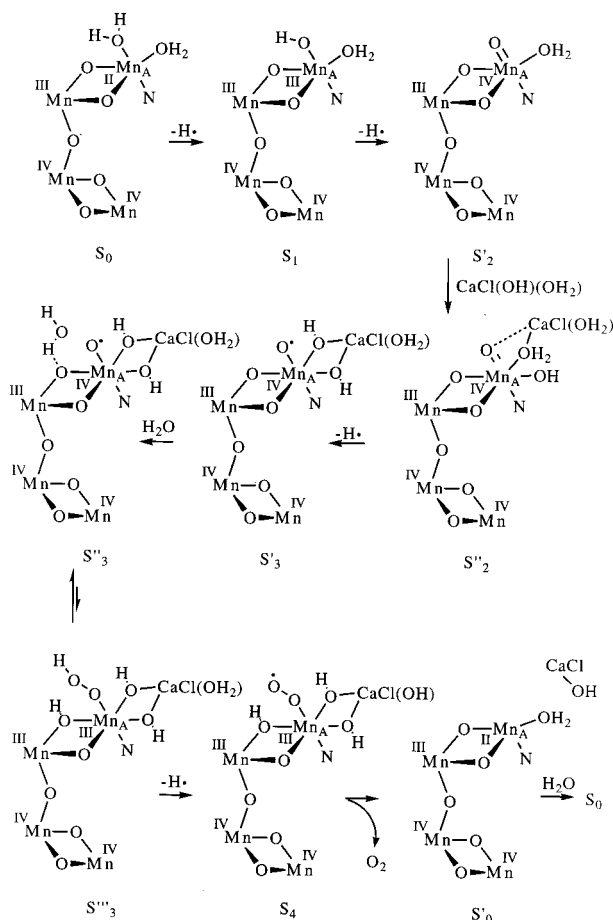

Figure 2. Proposed sequence of S states using the current standard[1a] tetramanganese structural model for the OEC. A similar series of S states should be possible whatever the exact structure of the Mn_4 cluster, however.
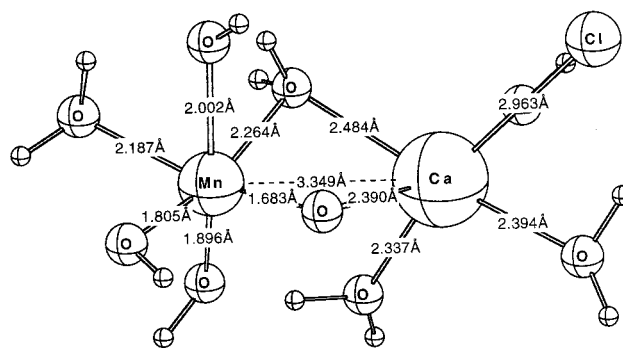

Figure 3. Structure of the chelated form of the S_2 state derived from the quantum chemical computations.

from the 3.4 Å in S_3. The paramagnet interacting with the Mn cluster in S_3, proposed in early work,[11] is identified here as the oxyl center. Finally, the $S_2 \rightarrow S_3$ step has the highest activation energy, consistent with the structural rearrangement postulated here.

The calculated energetics for the model species in Figures 2–9, shown in Table 1, agree reasonably well with the above assignment of S states. Of course, this model is quite simple and the energetics obtained from the B3LYP method are not exact. We estimate that tuning effects of the model (section IIIg) and more accurate calculations could be expected to change the energies by ± 5 kcal/mol. The first question studied in the present model is whether the S_3 oxyl state in Figure 5 can be reached by H abstraction from the bridging water in the chelating
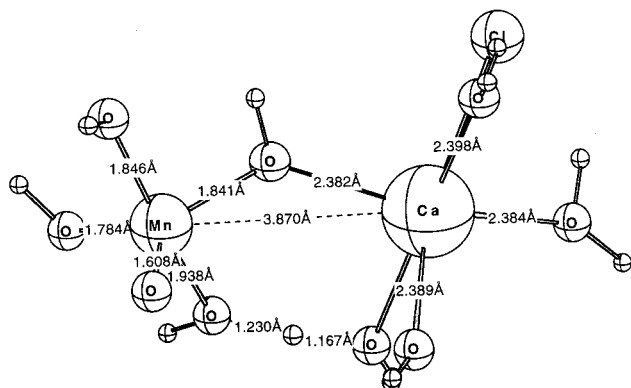
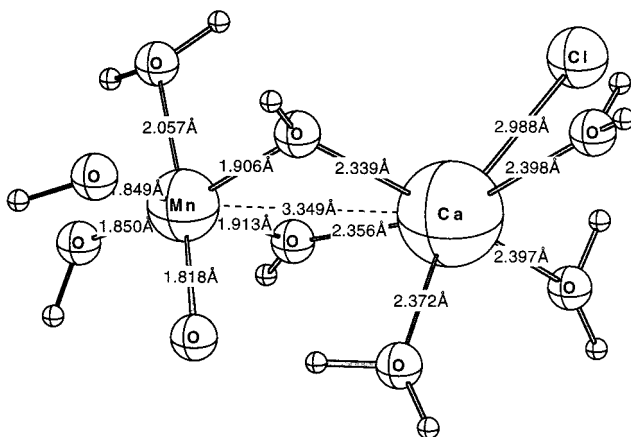**Figure 4.** Structure of the singly bridged isomer of the S_3 state with a 5-coordinate Mn(V)=O group.

Figure 5. Structure of the tightly chelated isomer of the S_3 state with a 6-coordinate Mn(IV)−O• derived from the quantum chemical computations.
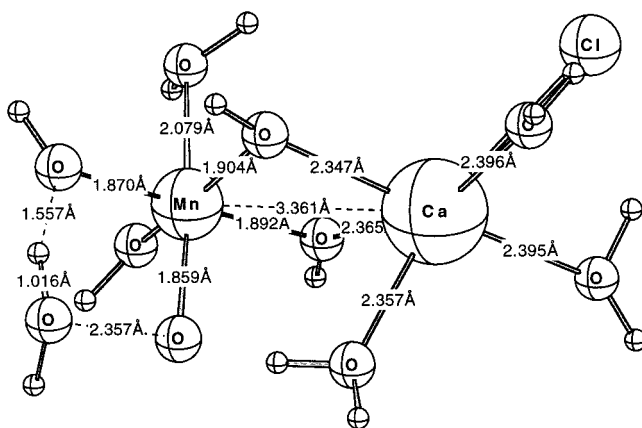

Figure 6. Structure of the tightly chelated isomer of the S_3 state with a 6-coordinate Mn(IV)−O• but including the outer-sphere water molecule required for the S_3 to S_4 conversion.

Figure 7. Structure of the transition state for the S_3 to S_4 conversion obtained as described in the text. A hydrogen atom of the outer-sphere water is abstracted by a Mn−OH group to leave an incipient hydroxyl radical in the outer sphere. The O···O bond of length 1.9 Å is newly forming between the outer-sphere incipient hydroxyl and the oxyl group bound to Mn. This is believed to be the rate-limiting step.

Figure 8. Structure of the S_3 state isomer having a 5-coordinate Mn-(III)−O−OH group, formed via the transition state of Figure 7. This isomer is expected to be slightly higher in energy than the resting state of S_3, but it lies on the proposed pathway to O_2.

Figure 9. Structure of the S_4 state after H-atom abstraction from the structure shown in Figure 8. O_2 has been formed and is weakly bound to the metal.

S_2 oxo state of Figure 3. The calculated O−H bond strength of this water is 88.6 kcal/mol, which is reasonably close to the tyrosine O−H bond strength of 86.5 kcal/mol. The next question concerns the relative energies of the three different S_3 structures shown in Figures 4−6. The calculated energy difference between the S_3 oxyl structure in Figure 5 and the singly bridging 5-coordinate S_3 oxo structure of Figure 4 is 0.3 kcal/mol in favor of the oxo structure. The S_3 MnO$_2$H structure is calculated to be 4.4 kcal/mol more stable than the oxyl structure. These energy differences are quite small, and the relative order could change using a different model (see subsection IIIg). In the S-state assignment above, the oxyl state is suggested to be the most
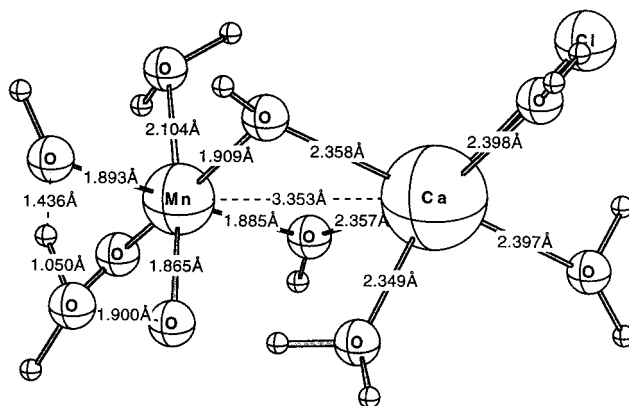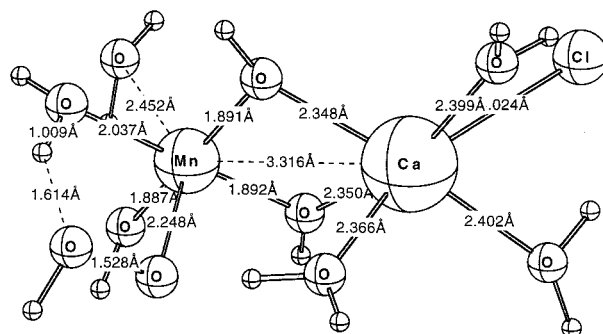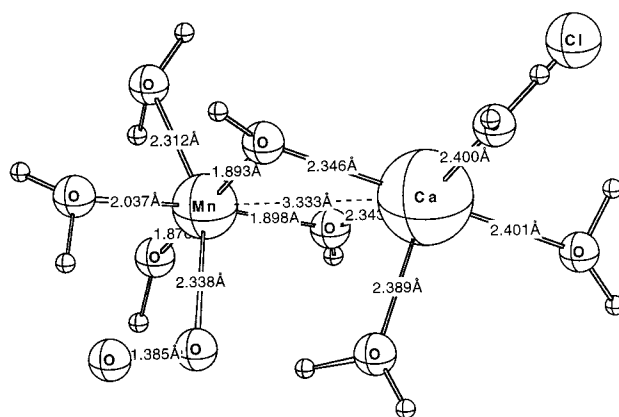
stable, but this is mainly based on accord with experimental observations, as already mentioned. The most critical aspect of the present model, the barrier for O−O bond formation, is discussed in the next subsection. Finally, the O−H bond strength of the hydrogen atom of the O$_2$H ligand in Figure 7 is calculated to be only 74.9 kcal/mol. This means that H abstraction by the tyrosine radical to reach the O_2 structure of Figure 9 should occur easily. However, in the enzyme, tuning effects (subsection IIIg) may make this O−H bond strength slightly stronger in order not to waste too much energy in this step.

Table 1. Relative Energies (kcal/mol) of the Key Species Discussed in This Paper

system	figure no.	energy (kcal/mol)
TyrOH		0^a
TyrO•		+86.5
S_2-oxo	3	−88.6
S_3-oxo	4	−0.3
S_3-oxyl	5, 6	0^a
O−O tr. state	7	+9.9
S_3-MnO$_2$H	8	−4.4
S_4-Mn(O$_2$)	9	+70.5

^{a} Reference states.

f. The Mechanism for O−O Bond Formation. It might seem that, with a route to the oxyl state in hand, finding the mechanism for O−O bond formation would be trivial. Almost any OH ligand might be expected to form an O$_2$H with the oxyl ligand via a low barrier, but this is not so. A large number of different attempts to form an O−O bond using the model oxyl complex in Figure 3 were made before a mechanism with a reasonably low barrier was found (Figures 3−9). In the first attempts, the oxyl ligand was simply brought closer to one of the OH ligands on manganese, varying the O−O distance in steps of 0.10 Å from 2.00 to 1.80 Å, without any noticeable tendency for O−O bond formation. The barriers were found to be >30 kcal/mol, independent of which OH ligand was used. The bridging OH ligands were no better than the terminal ones. Recall that the terminal OH ligands model μ-oxo groups, but these bridging groups could of course also be involved in O−O bond formation. Similar attempts with a 6-coordinate Mn monomer but without the Ca complex had very similar results.

In the next set of attempts a different model than the one in Figure 3 was used, with a bridging chloride instead of one of the hydroxyl groups. This allows the use of an OH ligand on Ca at the same place occupied by the chloride ligand in Figure 3. This hydroxyl ligand on Ca should be very nucleophilic and might be thought to be ideal for O−O bond-formation with the oxyl ligand. This was actually the original idea[12d,7b] which led to models in which manganese and calcium cooperate in the O−O bond-formation process. However, despite numerous attempts, no low-barrier route to an O−O bond was found via such a pathway. Part of the barrier was due to the non-negligible cost of bringing the Ca−OH ligand into reasonably close contact with the oxyl ligand on Mn, but in addition, the O−O bond-formation part did not occur easily. Part of the reason is that the Ca−OH group has to approach the oxyl group almost along the Mn−O bond direction. This is clearly not optimal since the oxyl group must have a singly occupied π-orbital (since the Mn−O π bond is broken) where nucleophilic attack would be easier.

Going back to the earlier attempts to form an O−O bond between the oxyl and an OH on manganese, we tried to conceive of a better pathway that would avoid deforming the octahedral ligand sphere around Mn. Introduction of an outer-sphere water molecule can do this. As mentioned above, the oxyl group is a π-radical, so just bending the oxyl toward the OH is not optimal. However, with an external water molecule, the orbital overlap is much more favorable. This extension of the model lowers the barrier, but not by enough in most cases. For a manganese monomer the barrier is still above 25 kcal/mol. Introducing still another outer-sphere water lowers the barrier to about 20 kcal/mol. Quite similar results were obtained if the chelating Ca complex is included in the model and one of the bridging hydroxyls is used. One reason the barrier is high in these

approaches is that the strongly bridging hydroxyl will turn into an only weakly bridging water group and a large part of the chelating energy is lost. Another reason the barrier is high is that the JT effect is important in the product Mn(III)−O$_2$H species. In these models, the JT axis with weakly binding ligands tends to form along the Mn−O$_2$H bond direction and the ligand trans to this one has to distort. One way to avoid the above effect of a loss of chelating power in the Ca complex is to involve one of the terminal hydroxyl groups on manganese instead. In the original idea of the model complex in Figure 3, these hydroxyls were intended to model μ-oxo bridges to the second manganese in the dimer. For low oxidation states, such as those in the Mn(III) product, these μ-oxo's are known to be quite basic which should help the O−O formation process. However, directly involving a μ-oxo bridge is not absolutely necessary since, in principle, this hydroxyl group could be a terminal hydroxyl in the real system, in which case one of the Mn−Ca bridging hydroxyls models a μ-oxo bridge instead.

When the O−O bond-formation step is modeled as in Figures 6 and 7, the O−O bond forms readily with a satisfactory barrier of only 9.9 kcal/mol. Dielectric effects from the surrounding protein have the very small effect of raising the barrier by 0.6 kcal/mol. Zero-point vibrational effects are also estimated to be very small. The calculated barrier is in line with experimental estimates of the barrier height in the step between S_3 and S_4.[22] This is the lowest barrier height for O−O bond formation obtained so far in any model.

One way of thinking of this key step is to consider that an H atom transfers from the second-sphere water to a terminal OH (modeling a μ-oxo group in the real cluster) to leave an incipient outer-sphere hydroxyl radical that couples with the reactive oxyl group, also having radical character. Polar effects are expected to favor facile O−O bond formation because the inner-sphere radical is expected to be electron deficient as a result of the presence of the adjacent Mn(IV) center and the incipient outer-sphere radical is expected to be electron rich. H-bond formation between a water bound to Ca and the Mn oxyl may facilitate the reaction by stabilizing the negative charge that is expected to build up on this oxygen in the transition state.

More recent work shows that the low-spin triplet surface can also lead to O−O bond formation, but in this case, too, the approach to the transition state still shows a very similar high-spin population on the oxygen as in the high-spin state and so it has strong oxyl character. We therefore expect the barrier will be low on the triplet surface only when the oxyl state is easy to achieve for the starting complex, as is expected to happen for a six-coordinate species.

g. Tuning Effects and the Formation of the O−O Bond. The model shown in Figures 3−9 is, of course, only the simplest possible one having the main properties necessary for it to conform with the general conditions discussed above. The most obvious extension of the model is to include also the other manganese atom in the dimer. This is more onerous computationally but will be done soon. Since, the μ-oxo bond is probably protonated in the O−O formation process (see Figures 8 and 9), the oxidation state of the other manganese might not be unimportant. A very strongly bound μ-oxo is not likely to be basic enough, and our suggestion is therefore that the other manganese is Mn(III), which leads to a suggested active Mn(III)−Mn(II) dimer in S_0. This suggestion is the same as in other leading models based on experimental information.[1a,5] The basicity of the μ-oxo bond should also to some extent depend

(22) Babcock, G. T. *Curr. Opin. Chem. Biol.* **1998**, 2, 244.

on the other ligands on the active manganese but could also depend on the orientation of a possible JT axis on the second manganese, which means that it could also depend on the detailed ligands in this part of the dimer.

The second major tuning parameter in the model is the choice of Mn−Ca bridging ligands. One of these has to be a hydroxyl group obtained by H abstraction from a water in going from S_2 to S_3. The main candidates for the other bridging ligand are a hydroxyl group as in Figure 3, a chloride, or a carboxylate ligand. When these bridges are used, the Mn−Ca distance in the tightly chelated complexes differs in a characteristic way. For a hydroxyl bridge $d(\text{Mn}-\text{Ca})$ is 3.3−3.4 A, for a chloride bridge $d(\text{Mn}-\text{Ca})$ is about 3.6 Å, and for a carboxylate bridge $d(\text{Mn}-\text{Ca})$ is 3.8−3.9 Å. For the weakly chelating stable oxo complexes the Mn−Ca distance is longer than 4.5 Å. All these choices of bridging ligands are presently being investigated in more detail. So far, the results using a bridging chloride have been quite unpromising. The barrier for O−O formation is raised by more than 7 kcal/mol, and the stabilization of the oxyl state is reduced compared to the weakly chelating oxo case. For the model complex in Figure 3, the total energy is also significantly increased if a chloride is bridging instead of a hydroxyl. This means that, in the models tried so far, the chloride does not play a significant role in the O−O formation process, consistent with the fact that several anions can replace chloride without any significant effects on the turnover rate. Chloride could simply be used because it is abundant and harmless, not interfering in any way with the O−O formation process.

The results using a carboxylate bridge are much more interesting than for the chloride bridge. Essentially the same low barrier for O−O formation is found as for the bridging hydroxyl. A carboxylate also appears to be particularly well suited to cause the rearrangement from 5- to 6-coordination required for oxyl formation, since it can reach different coordination sites on manganese quite well. A carboxylate bridge also has an advantage compared to other bridges in relation to the third major tuning parameter discussed below.

The third parameter discussed here is the choice of ligand trans to the oxyl. In the model in Figure 3 this ligand was chosen as water, but this leads to certain problems for this model at the chelation stage. For the loosely coupled Mn−Ca complex at S_2 where manganese is in an Mn(IV)=O oxo state, a proton transfer is found from this water to the hydroxyl on the Ca complex, which leads to the additional negative effect of the manganese becoming 4-coordinate, which in turn leads to an energy gain that would prevent the formation of the oxyl state. This problem can be removed by replacing the water ligand by an imidazole (histidine) ligand. However, for the bridging hydroxyl ligand, this causes an increase of the O−O barrier by about 7 kcal/mol. This increase does not occur for the bridging carboxylate ligand, which has essentially the same low barrier with a histidine as with a water trans to the oxyl. This result shows that many quite strongly coupled parameters are available for tuning optimal O−O bond formation, and it is now clear that the present investigation only represents the beginning of the investigation of the tuning phenomenon.

h. Limitations of the Model and Possible Tests of the Mechanism. The application of theoretical methods to the problem of enzyme mechanisms is in a very early stage of development, and we cannot yet be sure how well our simple quantum models represent the enzyme. Benchmarking of the quantum chemical methods employed, however, suggests that inadequacies in these methods are not the main factors limiting

the quality of the final results. The procedure we have adopted is to choose sufficiently simplified quantum model compounds to make the problem tractable and see whether the results obtained have promise. We believe that there are indeed sufficient points of contact between the theoretical results obtained and the known biophysical and bioinorganic data to provide a solid basis for further work along these lines. In this future work, we hope to establish how well the simplified models represent more complex clusters, for example, by adding further Mn atoms. Once better structural data are obtained on the OEC, better quantum models will automatically become available.

One difficulty with the mechanism of Figure 2 is the geometric relation of the tyrosyl radical to the hydrogens to be abstracted in the various steps. This aspect cannot be adequately addressed until structural data are available on the protein, however. We assume that the real system is so arranged to require minimal motion of this tyrosine.

The geometry of the Mn cluster is still not completely agreed, and that of Figure 2 is merely a candidate structure. The location of the calcium ion shown in Figure 2 is also speculative—the most recent Sr EXAFS data,[8b] which suggest the presence of two Mn···Ca vectors of 3.7 Å, could imply that the Ca is located in the "jaws" of the Mn cluster. Such a placement would not be expected to alter the conclusions presented here in any significant way.

Other problems may be quantitatively significant, such as the effects of the real versus model ligands on relative energies and any protein effects on the cluster, as well as many others, but these cannot be fully evaluated at present. The only way we can make progress is to refine the quantum chemical work and continue to make comparisons with the new biophysical and bioinorganic data as they become available.

A number of features of the present mechanism may be subject to experimental tests, however, the proposed motion of the Ca in going from S_2 to S_3, the presence of a Mn=O oxo in S_2, the involvement of an outer-sphere water molecule in $S_2 \rightarrow S_3$, and the redox activity of only one out of the four manganese ions of the cluster. The lower coordination number proposed for states S_0-S_2 may be a less useful test because of the possibility of weak "(5 + 1)"-coordination, as discussed earlier, but it should be possible to test some of the other structural predictions.

IV. Conclusion

We propose that spin considerations point to a high-spin oxyl Mn(IV)−O• state as the only group reactive enough to give a low-barrier O−O bond-formation reaction in photosynthetic oxygen evolution. A sufficiently reactive oxo cannot be formed directly by an H abstraction by a tyrosyl radical, however, and therefore an indirect pathway is suggested in which the role of the Ca cofactor is to stabilize the fully 6-coordinate state that turns on the necessary oxyl radical character in the Mn oxo at an appropriately late stage of the cycle ($S_2 \rightarrow S_3$).[23] This S_3 state can lead directly to O_2 if a second-coordination-sphere

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(23) The present mechanism does not absolutely require that the oxyl state be reached in the S_2 to S_3 step. However, if the oxyl state has not been reached at S_3, the O−O formation barrier has to be the sum of the excitation energy to reach the oxyl state and of the barrier on the oxyl state surface. Since the experimental barrier is low, about 10 kcal/mol, this means that the excitation energy to reach the oxyl state from S_3 has to be significantly less than 10 kcal/mol. Furthermore, the calculations give no indication of a pathway on the oxyl surface with an O−O formation barrier much less than 10 kcal/mol. Even if the oxyl is not the ground state in S_3, the oxyl is probably sufficiently low in energy so that state mixing can give the oxygen a significant radical character even in the ground state.

water molecule is pressed into service. An S-state cycle is proposed in which all the states are associated with appropriate structures for the Mn−Ca cluster. We also suggest that only one Mn atom of the OEC cluster is redox-active and that all the chemistry goes on at this site. The role of the ligand set in tuning the energies of the intermediates is also briefly discussed.