

# Probing the Mechanism of Water Oxidation in Photosystem II

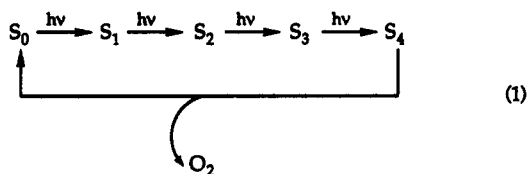
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Received June 3, 1991 (Revised Manuscript Received August 29, 1991)

Photosynthesis relies on a succession of apparently unlikely events, many of which are still poorly understood.<sup>1</sup> One of the most remarkable is the conversion of water into O<sub>2</sub>, which takes place in a membrane-bound enzyme complex, called photosystem II (PS II).<sup>2</sup> This process provides electrons to the oxidized pigment P<sub>680</sub><sup>+</sup> and so restores it to the photoactive form. The protons released in water oxidation contribute to the transmembrane proton gradient, subsequently tapped as an energy source by the plant.

Oxygen evolution is a four-electron oxidation. In a classic experiment, Joliot<sup>3</sup> showed that a train of light pulses had the effect of cycling PS II in such a way that a maximum in O<sub>2</sub> evolution was observed every four pulses. This implies that each pulse is associated with the production of a single electron hole or oxidizing equivalent and that PS II stores these holes until a sufficient number have been accumulated to release O<sub>2</sub>.<sup>4</sup> The successive S ("store") states run from S<sub>0</sub> to S<sub>4</sub> (eq 1). S<sub>4</sub> reverts to S<sub>0</sub> with the loss of a molecule of O<sub>2</sub>.



It has long been known that O<sub>2</sub> evolution in PS II requires Mn ions.<sup>5</sup> Only recently has evidence accumulated showing that a complex of four redox-active Mn ions constitutes Kok's redox store.<sup>6</sup> The active-site Mn cluster is probably either tetranuclear (Mn<sub>4</sub>)<sup>7</sup> or trinuclear with a nearby mononuclear site (Mn<sub>3</sub> + Mn).<sup>8</sup> Crystal structural data are not available for any O<sub>2</sub>-evolving PS II complex. We have therefore carried out biophysical studies of the natural system and bioinorganic studies of synthetic model complexes aimed at

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Holden Thorp began his scientific career as an undergraduate with T. J. Meyer at the University of North Carolina at Chapel Hill. His graduate work with Harry Gray at Caltech concerned the photochemistry of dioxorhenium(V). His postdoctoral work at Yale, some of which is described here, involved Mn models for photosystem II. He is now Assistant Professor at North Carolina State University, where he is a Presidential Young Investigator and a fellow of the David and Lucile Packard Foundation. His current research involves mechanistic studies of the oxidation of biomolecules by transition-metal complexes.

Robert Crabtree was born in London in 1948. After research with Malcolm Green at Oxford and Joseph Chatt at Sussex, he worked as Attache de Recherche in Hugh Felkin's laboratory of the CNRS Natural Products Institute at Gif sur Yvette, near Paris. Since 1977, he has been at Yale, where he is now Professor. Other areas of study in the Crabtree group are C-H activation, especially mercury-photosensitized reactions, dihydrogen complexes, and the bioinorganic chemistry of nickel. Dr. Crabtree has recently written a text on organometallic chemistry.

answering key questions in photosynthetic water oxidation.

Our studies have been aimed at both structural and functional aspects of water oxidation. We have used EPR studies to help determine the structure of the Mn cluster,<sup>7a,b</sup> and we and others, including Christou,<sup>9</sup> Armstrong,<sup>10</sup> Gorun,<sup>11</sup> Wiegardt,<sup>12</sup> Lippard,<sup>13</sup> and Pecoraro,<sup>14</sup> have made a number of synthetic Mn clusters that have helped in understanding the physical properties of the natural system. Studies of the photoactivated assembly of the cluster<sup>15</sup> have been complemented by the development of synthetic systems that increase their nuclearity upon oxidation in a manner related to the photoactivation process.<sup>16</sup> The Mn cluster binds ligands such as NH<sub>3</sub> late in the S-state cycle,<sup>17</sup> which suggested to us that structural rearrangements accompany cluster oxidation. We have looked at the effects of some possible rearrangements on the physical properties of synthetic clusters,<sup>18</sup> and

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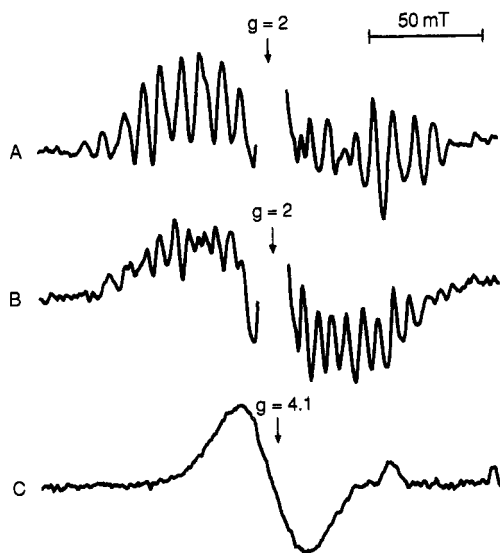
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**Figure 1.** Light-induced  $S_2$ -state EPR spectra from PS II membranes: (A) multiline EPR signal from an untreated sample; (B) multiline EPR signal produced in the presence of  $\text{NH}_4\text{Cl}$ ; (C)  $g = 4.1$  signal from an untreated sample. Reprinted with permission from ref 21. Copyright 1989 Elsevier.

we have studied in detail the thermodynamics and kinetics of one of these rearrangements, proton-coupled electron transfer, in detail.<sup>19</sup> In this Account, we present some of these studies and try to correlate the results on the natural system with work done on synthetic model complexes.

### Structure of the $\text{O}_2$ -Evolving Center in Photosystem II

Extended X-ray absorption fine structure (EXAFS) studies on the Mn ions have been reported.<sup>3,20</sup> Analyses of the EXAFS of the Mn ions in the  $S_1$  state indicate that there are 2–3 Mn...Mn separations of 2.7 Å and 1 Mn...Mn and/or Mn...Ca separation of 3.3 Å. The 2.7-Å distances are consistent with the presence of  $\text{Mn}(\mu\text{-O})_2\text{Mn}$  units, but EXAFS alone cannot distinguish between the  $(\text{Mn}_4)$  and  $(\text{Mn}_3 + \text{Mn})$  models. Fortunately, the cluster also exhibits characteristic EPR signals.

The EPR-active  $S_2$  state gives either a multiline signal at  $g = 2$  (Figure 1A) or a broad signal at  $g = 4.1$  (Figure 1C).<sup>21</sup> The initial discovery of the  $g = 2$  multiline EPR signal by Dismukes and Siderer<sup>22</sup> led to important contributions from the groups of Klein and Sauer,<sup>23</sup> Vännngård and Andréasson,<sup>2c,24</sup> and Rutherford.<sup>2d,25</sup>

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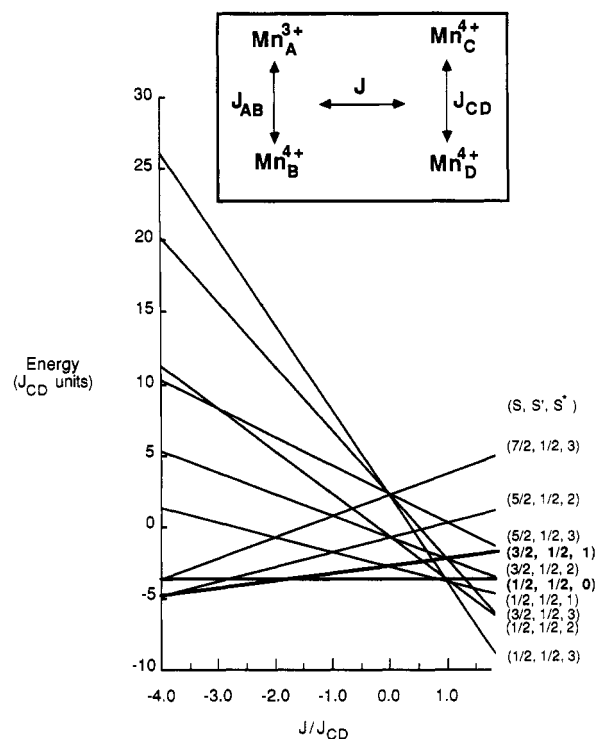
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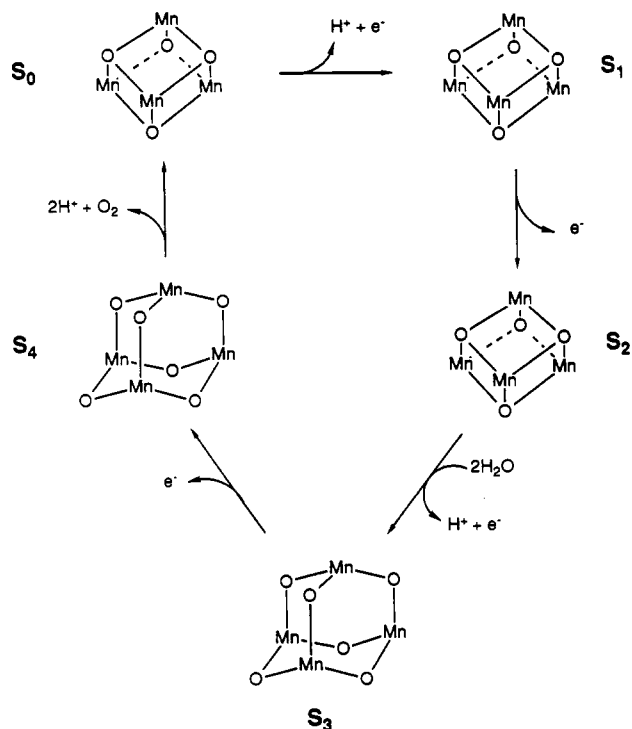
**Figure 2.** Lowest energy levels for an exchange-coupled  $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}_3$  tetramer in the limit where  $J_{\text{AB}}$  is large and antiferromagnetic. Reprinted with permission from ref 21. Copyright 1989 Elsevier.

The  $g = 2$  multiline and  $g = 4.1$  EPR signals are interconvertible, and both arise from Mn.<sup>7b,26</sup> In the  $(\text{Mn}_4)$  model, both of these signals arise from different spin states of the tetranuclear cluster, as we shall describe below. In the  $(\text{Mn}_3 + \text{Mn})$  model, the  $g = 2$  signal comes from an  $S = 1/2$  state of an EPR-active oxidation state of the trimer, which is in redox equilibrium with a Mn(III) monomer some distance away. The appearance and disappearance of each of the signals are assigned to a shift in the redox equilibrium that yields an EPR-silent trimer and a Mn(IV) monomer, which would be expected to give a  $g = 4$  signal from its high-spin  $d^3$  ( $S = 3/2$ ) ground state. As we shall see, a key point of the  $(\text{Mn}_3 + \text{Mn})$  model is that a monomer is responsible for the  $g = 4.1$  signal,<sup>24b</sup> which, when it was first observed, exhibited no hyperfine structure.

In our group, EPR studies were performed that led to the development of the  $\text{Mn}_4$  model.<sup>7a,b,21,26</sup> We were able to devise a vector-coupling scheme for a Mn tetramer that accounts for the observed EPR signals, as shown in Figure 2. Our model consists of a dimer-of-dimers structure with oxidation states of either  $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}_3$  or  $\text{Mn}^{\text{III}}_2\text{Mn}^{\text{IV}}_2$ . Both of these oxidation-state assignments are consistent with other physical data and are equivalent in our vector-coupling model. The exchange coupling in our model is such that we have two strongly antiferromagnetically coupled dimers ( $J_{\text{AB}}$  and  $J_{\text{CD}}$ ), and the two dimers are themselves ferromagnetically coupled ( $J$ ). Depending on the ratio  $J/J_{\text{CD}}$ , the ground state of the coupled system is either  $S = 1/2$  or  $S = 3/2$ . Thus, a structural rearrangement that alters the magnetic coupling only slightly could

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**Figure 3.** The adamantane-cubane mechanism for water oxidation. Reprinted with permission from ref 27. Copyright 1986 National Academy of Sciences.

cause the ground state to change from  $S = 1/2$  to  $S = 3/2$  and consequently change the EPR signal from the  $g = 2$  multiline to the  $g = 4.1$  signal. We felt that a likely tetranuclear structure that would give these couplings was a  $Mn_4O_4$  cubane-like complex that was distorted to give a dimer-of-dimers arrangement, and on the basis of this and some functional studies we will describe later, we proposed the mechanistic model for water oxidation shown in Figure 3.<sup>27</sup>

Recently, a key experiment was reported that seems to have resolved the nuclearity question.<sup>28</sup> Because PS II is a membrane protein, it is possible to prepare oriented samples of the membranes on Mylar sheets. These oriented samples exhibit a  $g = 4.1$  signal that shows resolved Mn hyperfine coupling. The number of hyperfine lines is  $\sim 20$ , which is too many for a monomeric Mn(IV) species.<sup>21</sup> Thus, the  $Mn_3 + Mn$  model fails to explain these new EPR data for the  $S_2$  state.

The exact arrangement of Mn ions in the tetranuclear array remains unknown, but based on the 2.7-Å Mn...Mn distances, oxo bridges are likely to be prevalent. The most relevant synthetic oxomanganese clusters exhibit adamantane ( $Mn_4O_6$ ),<sup>29</sup> cubane ( $Mn_4O_3Cl$ ),<sup>30</sup> and butterfly ( $Mn_4O_2$ ) structures,<sup>31,32</sup> and recently, Armstrong has prepared a series of complexes

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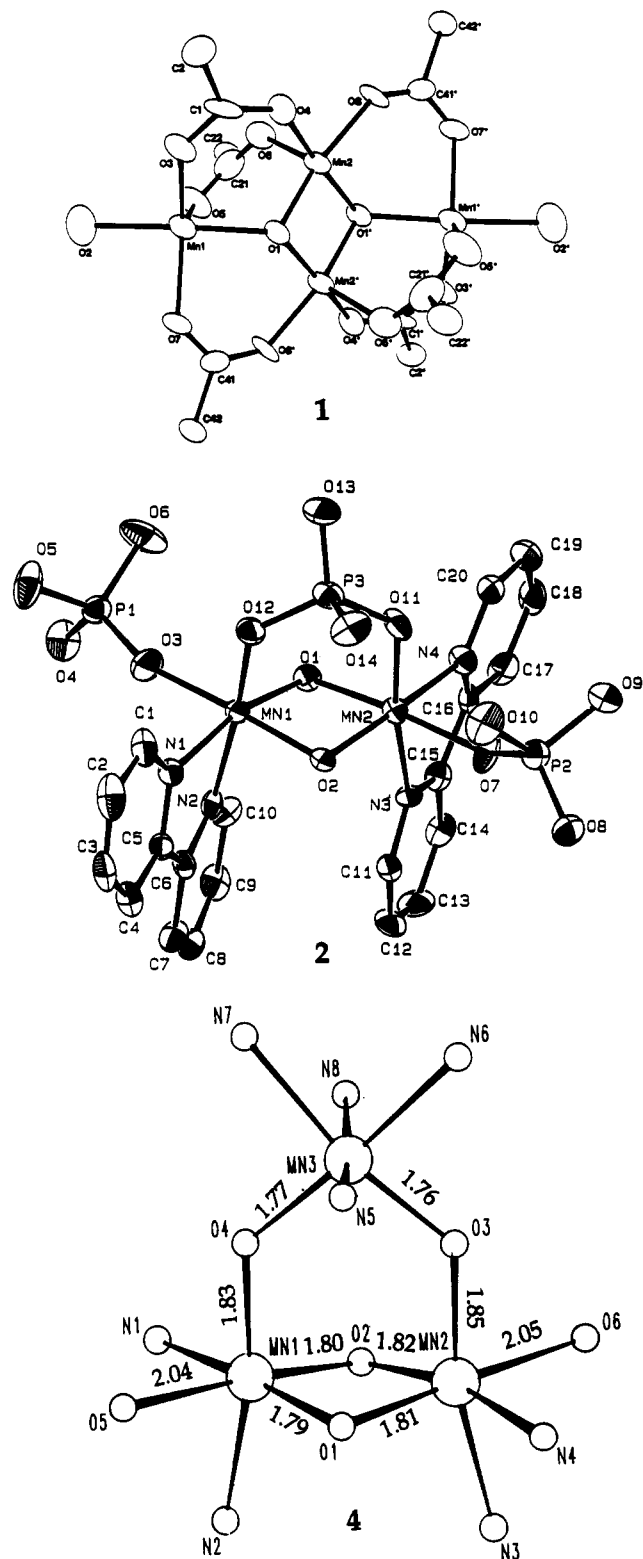
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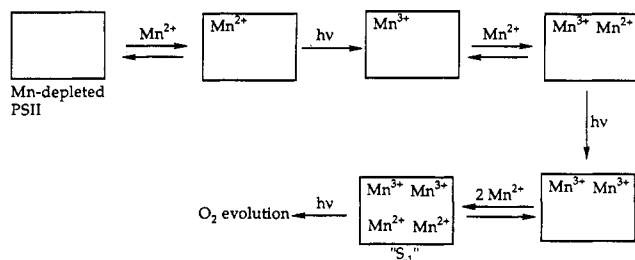
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**Figure 4.** ORTEP diagrams of  $Mn_4O_2(O_2CCPh_3)_6(OEt)_2$  (1),  $Mn_2O_2(bpy)_2(\mu-HPO_4)(H_2PO_4)_2$  (2), and  $Mn_3O_4(bpy)_4(OH)_2^{4+}$  (4).

having dimer-of-dimers-type structures.<sup>10</sup> We have proposed that the  $S_1$  state is best described by a distorted cubane structure ( $Mn_4O_4$ ), because it accounts for the number of 2.7-Å Mn...Mn distances. A distorted cubane structure has also been suggested by George, Prince, and Cramer<sup>20b</sup> as consistent with oriented EXAFS studies.

We have made some complexes that are also relevant to the structural questions. The tetranuclear butterfly



**Figure 5.** Scheme showing the fundamental processes of photoactivation of PS II.

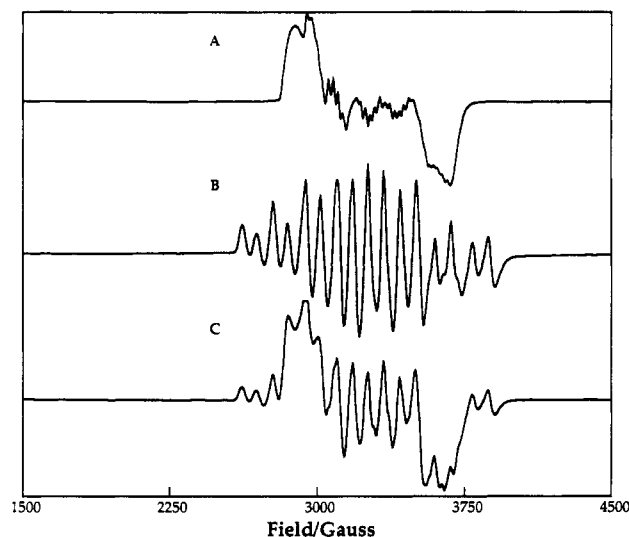
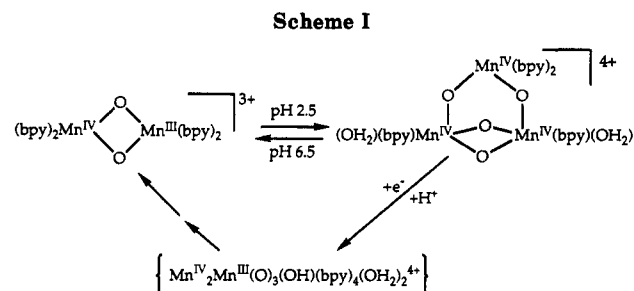
complex, **1** (Figure 4),<sup>32</sup> has two significant structural features. First, the complex is the first high-valent Mn tetramer to exhibit all-oxygen coordination, and recent studies on the natural system have shown that ligation to Mn is primarily by O donors.<sup>33</sup> Second, the cluster contains two five-coordinate Mn(II) centers. We have shown that these centers are relatively electron poor and appear by X-ray spectroscopy to be similar to Mn(III) centers.<sup>34</sup> This suggests that interpretation of X-ray spectroscopy on the natural system should take into account the possibility of unusual coordination states of Mn, such as five-coordinate Mn(II).

We have also prepared the phosphate-bridged complex **2** (Figure 4), which contains both terminal and bridging inorganic phosphate.<sup>35</sup> Phosphate is an excellent ligand to stabilize high-valent Mn and is oxidation resistant, but recent results suggest that coordination of phosphate to Mn in PS II is not a strong possibility.<sup>36a</sup> Nonetheless, the crystallization of **2** is relevant to enzymes known to contain phosphate ligation, such as purple acid phosphatase.<sup>36b-d</sup>

### Photoactivation

The photoactivation process is described in Figure 5. Cheniae's group first reported the reconstitution of Mn leading to O<sub>2</sub> evolution in purified, Mn-depleted PS II.<sup>37</sup> We have used EPR and O<sub>2</sub>-evolution measurements to characterize the intermediates formed in the photoactivation process.<sup>15</sup> A single Mn<sup>2+</sup> ion binds to the active site and is photooxidized to Mn<sup>3+</sup>; a second Mn<sup>2+</sup> is then bound. After a second photooxidation, two additional Mn<sup>2+</sup> ions bind to form a stable (II, II, III, III) tetramer, which we have associated with the "S<sub>1</sub>" state of the enzyme.

We looked for a model system in which oxidation of Mn in aqueous solution would produce a new cluster of higher nuclearity. In water, this normally leads to the precipitation of MnO<sub>2</sub>, a reaction that needs to be prevented. We found that acidification of an aqueous solution of the mixed-valent (III, IV) dimer [(bpy)<sub>2</sub>Mn(O)<sub>2</sub>Mn(bpy)<sub>2</sub>]<sup>3+</sup> (**3**) quantitatively produced an oxidized (IV, IV, IV) trimer, [Mn<sub>3</sub>O<sub>4</sub>(bpy)<sub>4</sub>(OH<sub>2</sub>)<sub>2</sub>]<sup>4+</sup> (**4**,



**Figure 6.** EPR spectra of (A) **4** in 0.5 M HNO<sub>3</sub>, (B) **3** in CH<sub>3</sub>CN, and (C) **4** in 0.05 M bpy buffer, pH 4.5. Reprinted with permission from ref 16. Copyright 1990 American Chemical Society.

Figure 4).<sup>16</sup> This complex is structurally analogous to Girerd's chloro analogue, [Mn<sub>3</sub>O<sub>4</sub>(bpy)<sub>4</sub>Cl<sub>2</sub>]<sup>2+</sup>, which was synthesized de novo in nonaqueous solution.<sup>38</sup> Our dinuclear-to-trinuclear rearrangement in water helps model photoactivation. Complex **4** also contains the first crystallographically characterized Mn<sup>IV</sup>-OH<sub>2</sub> group. We have successfully simulated the 35-line, *g* = 2 EPR spectrum of **4** in terms of a vector-coupling model containing two distinct exchange interactions. This has provided us with the opportunity to test on a synthetic complex the vector-coupling strategy used to analyze EPR spectra of the natural system.

Further experiments showed that **3** and **4** exist in a reversible equilibrium that is governed by redox potential and solution pH as shown in Scheme I.<sup>16</sup> Thus, low pH (<2.5) and oxidizing conditions favor **4**, and higher pH (>6.5) and reducing conditions favor **3**. This equilibrium is evident from electrochemistry and EPR, where both species can be detected at intermediate pH. For example, EPR spectra of **4** taken at pH 4.5 (Figure 6C) show that the solution clearly contains a mixture of **4** (Figure 6A) and **3** (Figure 6B). We have quantitated these signals and find that all of the Mn in solution is in the form of either **3** or **4**. Thus, the oxidation-induced assembly of higher nuclearity Mn clusters in aqueous solution, without the undesirable precipitation of MnO<sub>2</sub>, can occur in this system. This coupling of oxidation of Mn to a structural rearrangement that results in an increase in cluster nuclearity contributes to an understanding of the photoactivation process.

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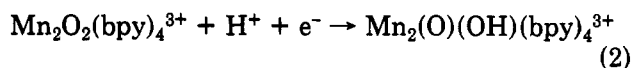
The mechanistic details of the conversion of 4 to 3 are also relevant. Electrochemical measurements show that the reduction of 4 is followed by a rapid chemical reaction that produces 3.<sup>16</sup> This reaction must involve the breaking and formation of a substantial number of Mn-oxo and Mn-aqua bonds; we estimate a lower limit of  $10 \text{ s}^{-1}$  for the rate of this reaction. None of the structural rearrangements proposed in our model (Figure 3) is as dramatic as this interconversion. The steps in Figure 3 are therefore likely to be kinetically feasible on the time scale of the water oxidation cycle.

### Coupling Structural Rearrangements to Mn Oxidation

**Ammonia Binding.** We have studied the binding of  $\text{NH}_3$  to the Mn cluster in PS II.<sup>17</sup> Earlier work was done by Izawa,<sup>39</sup> Velthuys,<sup>40</sup> and Yocum.<sup>41</sup> The binding of ammonia produces an altered multiline EPR signal, Figure 1B, which suggests that the binding is directly to Mn. Confirmation that the ammonia binds directly to Mn has come from electron spin-echo envelope modulation studies.<sup>42</sup> An interesting finding is that the binding of ammonia does not occur until after  $\text{S}_2$  has been produced,<sup>17</sup> which suggested to us that the binding of the substrate might occur late in the S-state cycle, as shown in our model (Figure 3). Since  $\text{NH}_3$  is a better nucleophile than water, we thought that it might bind earlier in the cycle than water, so we have proposed that water binds after the formation of  $\text{S}_3$  rather than  $\text{S}_2$ . This is also consistent with the observation that a much higher temperature is required for the  $\text{S}_2 \rightarrow \text{S}_3$  transition than the  $\text{S}_1 \rightarrow \text{S}_2$  transition.<sup>43</sup>

**Proton-Coupled Electron Transfer.** The results of the studies of ammonia binding to Mn in PS II got us interested in studying the effects of structural rearrangements on the properties of synthetic Mn clusters. In the natural system, release of a proton occurs in the  $\text{S}_0 \rightarrow \text{S}_1$  and  $\text{S}_2 \rightarrow \text{S}_3$  transitions (Figure 3).<sup>44</sup> We have suggested that proton-coupled oxidation occurs in PS II,<sup>19</sup> as has also been suggested in other oxo-bridged metalloenzymes such as hemerythrin.<sup>45</sup> The effect of proton coupling is that deprotonation of the oxo group will increase the stabilizing influence of the bridging ligand toward higher oxidation states; both the oxidized and reduced forms are thereby in their thermodynamically favored state of protonation. This also provides a subtle control mechanism for the enzyme: regulating the effective pH in the active site will tune the thermodynamics, and providing an efficient mechanism for the proton transfer will offer kinetic control.

We showed that complex 3 exhibits a quasi-reversible reduction whose potential varied by 59 mV/pH unit, according to eq 2 (Figure 7).<sup>19a</sup> This assignment is



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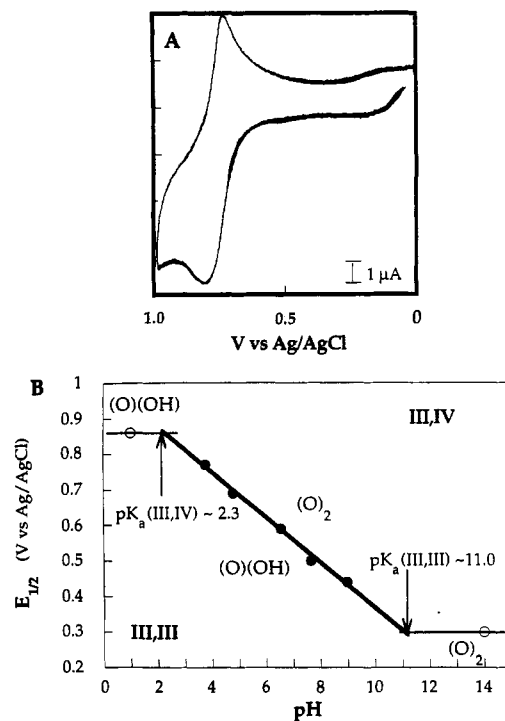
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**Figure 7.** (A) Cyclic voltammogram of 3 in 0.1 M phosphate, pH 3.78. (B) Pourbaix diagram for complex 3. Reprinted with permission from ref 19a. Copyright 1989 American Chemical Society.

consistent with the electrode kinetics; similar rate constants were observed for proton-coupled reductions of oxoruthenium complexes.<sup>46</sup> We have also measured an isotope effect for reaction 2,  $k_{\text{H}}/k_{\text{D}} = 4.6$ .<sup>19d</sup>

The proton coupling is sensitive to the nature of the ancillary ligands and the electrode surface. We found that replacement of two bpy ligands with the more strongly donating ligands in  $\text{Mn}_2\text{O}_2(\text{bispicen})_2^{3+}$  (bispicen = bis( $N,N'$ -2-pyridylmethyl)ethanediamine) causes the electron and proton transfers to decouple kinetically, resulting in a mechanism whereby electrochemical reduction precedes a separate protonation step.<sup>19c</sup> From the pH dependence of the cyclic voltammetry, we were able to estimate a  $\text{pK}_a$  for the (III,III) form of 8.3. The quasi-reversible kinetics for 3 shown in Figure 7 are observable only at activated glassy carbon, edge-oriented pyrolytic graphite, and tin-doped indium oxide working electrodes,<sup>19b</sup> an observation similar to those made in proton-coupled reactions of aqua-ruthenium complexes.<sup>46</sup>

**Binding of Water.** In our model, we propose that water binds to the cubane-like cluster in the  $\text{S}_2 \rightarrow \text{S}_3$  transition to form an adamantane-like cluster (Figure 3). The  $g = 2$  multiline EPR signal disappears upon the conversion of  $\text{S}_2$  to  $\text{S}_3$ , consistent with the oxidation of Mn to form an integer-spin-state species. However, X-ray absorption near-edge spectroscopy (XANES) studies show that the K-edge energy of Mn does not shift in this transition,<sup>47</sup> which might be expected from an alternative proposal that it is not Mn but rather a nearby histidine residue that is oxidized in the  $\text{S}_2 \rightarrow \text{S}_3$  transition.<sup>48</sup> Two lines of evidence imply that some sort

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of structural rearrangement occurs in  $S_2 \rightarrow S_3$ : the EXAFS of Mn in  $S_2$  and  $S_3$  are different, whereas the EXAFS of  $S_1$  and  $S_2$  are very similar,<sup>47</sup> and the  $S_2 \rightarrow S_3$  transition occurs only at or above 240 K, a temperature high enough to permit such a structural rearrangement.<sup>43</sup> Oxidation of Mn together with binding of a good donor ligand, such as water, in  $S_2 \rightarrow S_3$  could account for the failure of the Mn K-edge energy to shift, the change in EXAFS, the loss of the EPR signal, and the requirement for higher temperatures for the  $S_2 \rightarrow S_3$  transition.

**O-O Bond Formation.** The most dramatic and important step in the S-state cycle is the one about which the least is known. From studies on the natural system, the rate-limiting step is the formation of  $S_4$ , for which  $Ca^{2+}$  is required.<sup>49</sup> In the absence of  $Ca^{2+}$ , an altered  $S_3$  state is produced that cannot be oxidized to  $S_4$ , implying that a very special structure may be needed. In our model, we have proposed that two bridging oxo ligands in  $S_4$  couple to form a  $\mu_2$ -peroxo linkage.<sup>27</sup> Recently, Wieghardt has succeeded in crystallizing a dinuclear Mn(IV) complex containing such a bridge.<sup>50</sup>

Other proposals regarding O-O bond formation have been advanced. Lippard has suggested that  $\mu_2$ -oxo groups in two adjacent bis( $\mu$ -oxo)dimanganese units couple to form a  $\mu_4$ -peroxo  $S_4$  state.<sup>51</sup> While such a linkage has not been observed in a Mn complex, an iron complex containing a  $\mu_4$ -peroxo bridge of this type has been crystallized. Armstrong has proposed that O atoms from a  $\mu_2$ -oxo and a hydrogen-bonded terminal aqua ligand couple to form the O-O bond in  $S_4$ .<sup>10</sup>

In spite of considerable effort, no synthetic Mn clusters are known to catalyze water oxidation efficiently. Meyer has found that a dinuclear Ru complex,  $[(bpy)_2(OH_2)Ru^{III}ORu^{III}(OH_2)(bpy)_2]^{4+}$ , is a catalyst for water oxidation.<sup>52</sup> The oxidation of the aqua ligands

occurs by proton-coupled oxidation steps to form a Ru(V) dimer containing two terminal oxo ligands. The  $O_2$ -evolution step is proposed to involve the coupling of the adjacent terminal oxos to form a  $\mu_2$ -peroxo complex that liberates  $O_2$  before binding two new water molecules.

The formation of terminal oxo ligands on Mn seems unlikely, considering the facility with which oxo bridges are produced in polynuclear Mn complexes; no polynuclear Mn complexes containing a terminal oxo ligand are known. Additionally, the highest oxidation state of Mn in PS II is probably Mn(IV), for which no terminal oxo complexes of any sort have been isolated. From the biophysical evidence and the known coordination chemistry of high-valent Mn, it seems most likely that the O-O bond is formed from bridging oxo ligands, as we and others have suggested.

## Conclusions

Although we still have some way to go before we have a satisfactory understanding of the problem, we are beginning to see how the Mn cluster of the  $O_2$ -evolving center catalyzes water oxidation. In order for an O-donor environment to stabilize the high-valent Mn in  $S_3$  and  $S_4$ , it must be rich in the strongly donating  $O^{2-}$  ligands. For the same system to be stable in the relatively reduced  $S_0$ - $S_2$  forms, the oxo groups are probably protonated and the cluster may have rearranged to a different cluster geometry. Proton-coupled steps are probably required for transitions between the S states. Water is probably incorporated as terminal  $H_2O$  or bridging OH, but the proton-coupled oxidation leads to the production of oxo groups which finally couple to form  $O_2$ . A similar process may go on in photoactivation, but in this case the oxidation step leads to incorporation of additional Mn ions into the cluster.

*We thank all our co-workers who have been involved in this work, in particular Warren Beck, Jeff Bocarsly, Julio de Paula, Jennifer Innes, Bob Kulawiec, Rajesh Manchanda, Anne-Frances Miller, Joe Sarneski, Gayle Schulte, and Lynmarie Thompson. We acknowledge the continuing support of the National Institutes of Health and the United States Department of Agriculture.*

**Registry No.**  $H_2O$ , 7732-18-5.

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