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Photosynthetic Oxygen Evolution: Changes in Magnetism of the Water-Oxidizing Enzyme

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Abstract: Changes in magnetic susceptibility produced by single-turnover flashes of light have been measured for the first time for four of the oxidation states, so-called S states, produced during oxygen evolution in Photosystem II (PSII) complexes of spinach. The data reveal new insights into the structure and bonding of the manganese cluster responsible for catalysis of water oxidation. In samples that have been dark adapted for 15 min or longer to favor population of the "resting" S_1 state, a train of six flashes increases the paramagnetism on flashes 1, 3, and 5, while no or small increases are observed on flashes 2, 4, and 6. Advancement to the S_1 state does not restore the dark level of S_1 magnetism. This is due to two effects: formation of net paramagnetism from O_2 release on the $S_4 \rightarrow S_0$ reaction (scavengeable by glucose oxidase) and a large increase in magnetism for the S_1(resting) $\rightarrow$ S_2 reaction, which is not restored without dark readaptation. Comparison of these data with models proposed for the structure of the manganese site reveals that models in which oxidation of substrate water occurs prior to S_4 or oxidation of magnetically isolated Mn ions cannot account for the susceptibility changes observed. The large increase of 17 $\mu_B{}^2$/PSII observed for the S_1(resting) $\rightarrow$ S_2 oxidation is opposite in sign to the decrease in paramagnetism reported for oxidation of synthetic Mn dimers containing the μ_2-oxo-di-μ_2-carboxylato and di-μ_2-oxo-μ_2-carboxylato bridges undergoing the oxidation Mn_2(III,III) $\rightarrow$ Mn_2(III,IV). Consequently, these complexes must not provide complete structural representations of the bridging geometry or ligand types in the enzyme. The increase in susceptibility can be understood in terms of reduced antiferromagnetic coupling within a higher nuclearity cluster of three or four magnetically interacting Mn ions. This nuclearity is consistent with earlier EPR data.

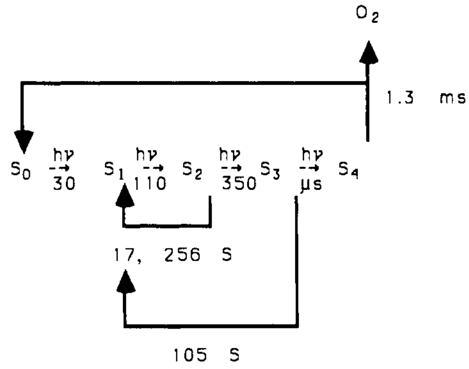
Photosynthesis in plants and algae produces the bulk of atmospheric oxygen through the oxidation of water. The oxygen gas so produced sustains all aerobic life as we know it on earth. A common biochemical apparatus appears to be responsible for catalyzing this fundamental process, from the most primitive cyanobacteria ("blue-green algae", dating back (2–3) × 10^9 years) to all known plants past and present. The biochemical apparatus has two parts, a photoactive reaction center membrane–protein complex termed Photosystem II (PSII) and a tightly associated water-oxidizing complex.[1-3] The reaction center protein complex binds the special chlorophylls and other chromophores needed for light absorption and charge separation. The primary photochemical reaction produces a strong oxidant that is not capable of directly catalyzing the four-electron oxidation of water (eq 1).

$$2H_2O \xrightarrow{\text{light + chloroplasts}} O_2 + 4e^- + 4H^+ \qquad (1)$$

Instead this is accomplished by a complex comprised of four manganese ions believed to be associated in the form of a ligand-bridged cluster of 2–4 ions that serve as a storehouse for the oxidizing equivalents and as the apparent substrate binding site. Essential roles are played also by chloride and calcium ions, but these have been less well characterized. The process appears to involve a concerted four-electron oxidation reaction, as suggested not only by the recovery in the yield of O_2 evolved every fourth flash in response to a train of short pulses of light[4,5] but also by flash-induced changes in manganese oxidation state as seen by EPR[6] and the exchangeability of isotopically labeled substrate water with free water in all oxidation states prior to the final state that releases O_2.[7]

There are five intermediate oxidation states in the catalytic cycle, called S states, S_0 through S_4, after Kok (Scheme I).[5] These are characterized by (1) an initial population of S_1/S_0 = 75%/25% in dark-adapted chloroplasts, (2) release of O_2 only on the $S_4 \rightarrow S_0$ reaction, and (3) a gradual loss of synchronization in the populations caused by random misses (10%) and double hits (5%) during flash photolysis. The half-times of the forward photochemical reactions and of the deactivation reactions in the dark are given in Scheme I.[8-10] Because S_1 is stable in the dark, while S_2 and S_3 decay to S_1 over tens of seconds, all S states except S_4 can be prepared readily with short pulses of light.

Scheme I



Knowledge about the structure and bonding of the manganese ions has come primarily from spectroscopic techniques and is far from complete. EPR spectroscopy on the S_2 state has established that the Mn spins are electronically coupled to give an $S = {}^1/_2$ ground state, while the other S states are EPR silent.[11] We therefore have sought to obtain information about the oxidation states and bonding of the manganese ions in *each* of the S states by measuring changes in their magnetic susceptibility. As a nonresonance technique, magnetic susceptibility can detect all

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(1) Brudvig, G. W. *J. Bioenerg. Biomembr.* **1987**, *19*, 91–104.
(2) Babcock, G. T. In *New Comprehensive Biochemistry* Amesz, J., Ed.; Elsevier North-Holland Press: Dordrecht, 1987; pp 125–158.
(3) Dismukes, G. C. *Photochem. Photobiol.* **1986**, *43*, 99–115.
(4) Joliot, P.; Barbieri, G.; Chabaud, R. *Photochem. Photobiol.* **1969**, *10*, 309–329.
(5) Kok, B.; Forbush, B.; McGloin, M. *Photochem. Photobiol.* **1970**, *12*, 457–475.
(6) Dismukes, G. C.; Siderer, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 274–278.
(7) Radmer, R.; Ollinger, O. *FEBS Lett.* **1986**, *195*, 285–289.
(8) Renger, G.; Weiss, W. *Biochim. Biophys. Acta* **1986**, *850*, 184–186.
(9) Dekker, J. P.; Plijter, J. H.; Ouweehand, L.; Van Gorkum, H. J. *Biochim. Biophys. Acta* **1984**, *767*, 176–179.
(10) Seibert, M.; Lavorel, J. *Biochim. Biophys. Acta* **1983**, *723*, 160–168.
(11) For a possible exception to this, see: Sivaraja, M.; Dismukes, G. C. *Biochemistry* **1988**, *27*, 6297.

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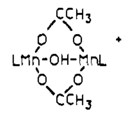
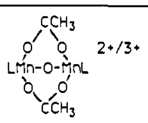
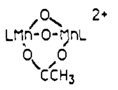
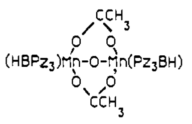
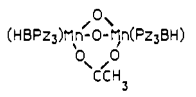
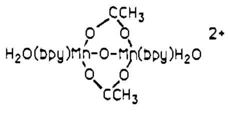
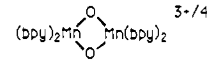
Table I. Summary of the Magnetic Data on Binuclear Manganese Complexes[a]

 $Mn^{II}Mn^{II}$ $J = -18\ cm^{-1}$ Refs:24, 25	 $n = 2\ (Mn^{III}Mn^{III})$ $J = +18\ cm^{-1}$ $n = 3\ (Mn^{III}Mn^{IV})$ $J = -80\ cm^{-1}$	 $Mn^{III}Mn^{IV}$ $J = -440\ cm^{-1}$
Ref: 26, 27	 $Mn^{III}Mn^{III}$ $J = -0.3\ cm^{-1}$	 $Mn^{III}Mn^{IV}$ $J \sim -300\ cm^{-1}$
Refs: 15, 28	 $Mn^{III}Mn^{III}$ $J = -6\ cm^{-1}$	 $n = 3$, $Mn^{III}Mn^{IV}$ $J = -300\ cm^{-1}$ $n = 4$, $Mn^{IV}Mn^{IV}$ $J = -288\ cm^{-1}$

[a] The derived Heisenberg exchange interaction constant for the interaction energy $H = -JS_1 \cdot S_2$. Negative J is antiferromagnetic coupling. All cations are ClO_4^- or BF_4^- salts; L = *N,N',N''*-trimethyl-1,4,7-triazacyclononane; $HBPz_3$ = hydrotris(pyrazoyl)borate; bpy = 2,2'-bipyridine.

paramagnetic species and can be used at temperatures where the samples are completely functional. For isolated paramagnetic ions, magnetic susceptibility is capable of detecting either changes in oxidation state or changes in spin state due to a change in ligand field strength. For clusters of paramagnetic ions, electronic interactions within the cluster often produce a coupling between the spins, which alters the observed magnetic susceptibility. The strength of this spin coupling can be quite sensitive to small changes in structure.

Thus measuring the susceptibility changes of the Mn ions during the S-state cycle could potentially provide structural and electronic information about the catalytic site. However, it has only recently become possible to measure such small and transient magnetic changes, by using ultrasensitive superconducting SQUID magnetometers. Here we demonstrate the first such measurements on O_2-evolving samples from spinach.

Results

The magnetic susceptibility instrument we are using was specifically designed and built by one of us (J.S.P.) for studying transient phenomena and to maximize sensitivity for samples at physiological temperatures. It is based on techniques and principles described previously.[12] First we present some data on a simple well-understood system that illustrate the techniques we are using and demonstrate our ability to measure small magnetic changes with excellent time resolution.

Figure 1 shows the results from laser photolysis of a solution of (carbonmonoxy)myoglobin (MbCO) at 20 °C. At $t = 0$ a laser pulse (<500-ns duration) completely dissociates all the CO bound to the heme iron, converting the sample to deoxyMb. With this change in ligation, the iron remains ferrous but changes from the low-spin $S = 0$ state to the high-spin $S = 2$ state, producing an increase in paramagnetism during the laser pulse. The data indeed show an increase in paramagnetism, with a rise time limited in this case by a 3-μs time constant low-pass filter imposed on the SQUID output. (It is worth noting that the paramagnetic resonance of even-spin systems such as high-spin ferrous iron is not detectable with microwave band (1–50 GHz) EPR spectrometers.)

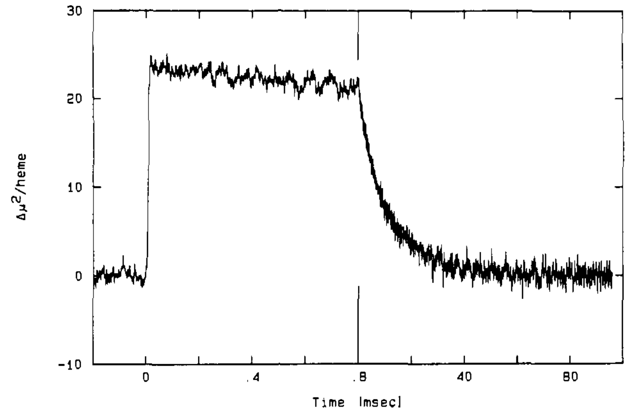


Figure 1. Changes in magnetism upon laser photolysis of a solution of sperm whale MbCO. Conditions: 250-μL sample, 120 μM [heme], 10% CO/90% nitrogen average of 20 repetitions, 3-μs time constant filter, applied magnetic field of 0.23 T. Note the break in the time axis.

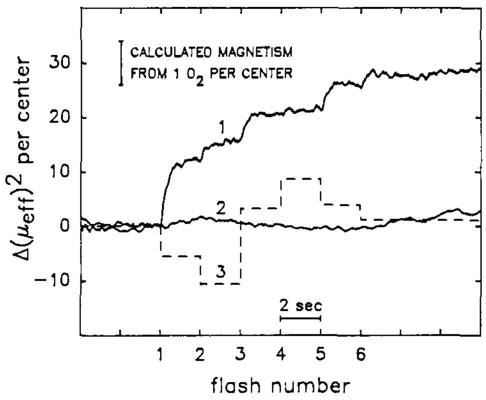


Figure 2. Curve 1, changes in the magnetism upon laser photolysis (six flashes, 2-s interval, and 0.5-μs duration) of O_2-evolving reaction center core complexes of spinach. Curve 2, same sample as curve 1 except the sample was inactivated by washing with 2 mM NH_2OH to remove 3–4 Mn^{2+} and resuspended in buffer including 1 mM benzidine and 1–2 mM DCBQ. Curve 3, calculated change in $\Delta\chi$ for oxidation of four independent Mn^{3+} ions, per the text. Chl = 0.4–0.7 mg/mL; buffer: 20 mM MES, 10 mM $CaCl_2$, 15 mM NaCl, 0.4 M sucrose, pH = 6.1, 1–2 mM DCBQ, T = 273 K; 0.3-s time constant filter.

By using the absolute calibration of the instrument[13] and the number of moles of myoglobin in the sample, the raw data have been converted to a scale showing the change in the squared effective magnetic moment ($\Delta\mu_{eff}^2$), in units of squared Bohr magnetons per heme, using the relation $\chi = N(\mu_{eff}^2)/3kT$. The spin-only moment of an isolated ion with spin S is $\mu_{eff}^2 = 4S(S + 1)\mu_B^2$, and thus the observed increase of 24 μ_B^2 is exactly that expected for an $S = 0$ to $S = 2$ transition. After the photolytic pulse, the CO begins to recombine with the myoglobin in a second-order reaction, returning the sample to its initial state, and these magnetic data are fully consistent with the known rate constant for this process.

Figure 2 (curve 1) shows the magnetic susceptibility changes created by a train of six laser pulses given to a sample of dark-adapted, O_2-evolving Photosystem II core complexes (obtained by extraction of spinach Photosystem II membranes with the detergent octyl β-D-glucopyranoside (OGP)).[14] The data reveal increases in paramagnetism after flashes 1, 2, 3, 5, and 6 and no change on flash 4. These data are technically much more difficult to obtain than the MbCO data, because the signals are nearly 2 orders of magnitude smaller and because we cannot use extensive signal averaging. Therefore, we have used a 300-ms time constant

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[12] Philo, J. S.; Fairbank, W. M. *Rev. Sci. Instrum.* **1977**, *48*, 1529–1536.

[13] Philo, J. S.; Dreyer, U.; Schuster, T. M. *Biochemistry* **1984**, *23*, 865–872.

[14] Ghanotakis, D. F.; Demetriou, D. M.; Yocum, C. F. In *Prog. Photosynth. Res., Proc. 7th Int. Cong. Photosynth.* Biggins, J., Ed.; Martinus-Mijhoff: Dordrecht, 1987; Vol. I, pp 681–684.

filter on the output to improve the signal/noise ratio. The signal rise times were still instrument limited with a 10-ms filter (not shown), which is consistent with the faster kinetics for photooxidation of the S states (Scheme I). The dark adaptation time between flash trains needed to restore the same pattern of changes seen in Figure 2 was about 15 min. This recovery time allows for reequilibration of the S states to their dark populations and has been confirmed by using the EPR signal from the S_2 state. The data are given in terms of $\Delta\mu_{\text{eff}}^2$/PSII, where we have normalized to the total Photosystem II concentration, based on the measured total chlorophyll concentration by assuming 70 Chl/PSII.[14] This includes all Photosystem II centers, even those that are inactive in O_2 evolution; hence the absolute values will be an upper limit. In order to observe the oscillations in Figure 2 the samples must also contain an electron acceptor. We have used dichlorobenzoquinone (DCBQ) to replace the plastoquinone lost during purification.[14] We have also verified that the first-flash signal amplitude increases linearly with sample concentration at and below that which was used to obtain the data in Figure 2. It was also verified that the signal amplitude could be light saturated. These controls ensure that all centers that can undergo photooxidation have received a photon.

The magnetic susceptibility changes are due to electron transport through Photosystem II, as shown by their disappearance upon incubation with the herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU; not shown) after all but the first flash. The bulk of these changes can be attributed to turnover of the water-oxidizing complex, as judged by the effect of washing the sample with the potent inhibitor hydroxylamine, which is known to release 3–4 Mn^{2+} ions from the complex. This treatment leaves the reaction center intact, and electron transfer from an artificial donor such as benzidine or diphenylcarbazide to an electron acceptor such as DCBQ or (dichlorophenyl)indophenol, respectively, does not restore any stable changes in $\Delta\chi$ (Figure 2, curve 2). This indicates that the contribution to the magnetic susceptibility arising from formation of the dichlorobenzosemiquinone is too small to observe, perhaps because of dissociation and disproportionation on a time scale faster than the 300-ms time constant.

Discussion

There are several interesting features of the susceptibility data. First, the magnitude of the first flash increase is very large, corresponding to a change of 10–12 μ_B^2/PSII in replicate samples. If spin-only magnetism of isolated ions were considered ($\mu_{\text{eff}}^2 = 4S(S+1)\mu_B^2$), this would correspond to the formation of 3.3–4 spin $S = {}^1/_2$ paramagnets. The calculated $\Delta\chi$ for the manganese site for the $S_1 \rightarrow S_2$ transition is predicted to be even larger at 17 μ_B^2/PSII if we make two assumptions: the usual Kok parameters of α = 10% misses and β = 5% double hits, 100% S_1 in the dark (this is compatible with the Kok formulation of 75% S_1 and 25% S_0 because 25% of the centers have a reduced accessory donor D in dark-adapted samples), and the semiquinone form of the photoreduced acceptor, $DCBQ^-$, is unstable in solution and disproportionates to diamagnetic products in a time shorter than the 0.3-s time constant. The former of these has been confirmed by using the S_2 EPR signal intensity formed by a train of laser flashes.

The second interesting feature of the data is that it is impossible to account for the progressive increases in χ seen in Figure 2 by any model in which there is sequential one-electron oxidation of free substrate water on each flash (eq 2). The predicted $\Delta\chi$ are too small and have a very different pattern than the experimental results. This confirms what is already known in a limited way from other experiments that S-state advancement is not due to oxidation of free water prior to reaching S_4.

$$2H_2O \xrightarrow[\Delta\mu_{\text{eff}}^2=+3]{} OH + H_2O \xrightarrow[\Delta\mu_{\text{eff}}^2=-3]{} H_2O_2 \xrightarrow[\Delta\mu_{\text{eff}}^2=+3]{} HO_2 \xrightarrow[\Delta\mu_{\text{eff}}^2=+5]{} O_2 \quad (2)$$

The experimental data also disprove any model in which four independent Mn^{2+} or Mn^{3+} ions are sequentially oxidized, since this would decrease χ on every step prior to substrate oxidation (the electronic spins for high-spin Mn^{2+}, Mn^{3+}, and Mn^{4+} are $S = {}^5/_2$, 2, and ${}^3/_2$, respectively). This is illustrated in Figure 2 (curve 3), which gives the predicted $\Delta\chi$ for successive independent one-electron oxidations of 4 Mn^{3+} high-spin ions (starting in the S_1 state and using the Kok parameters α = 10%, β = 5%). This includes the contribution to the paramagnetism from S = 1 oxygen and $S = {}^1/_2$ D to D^+ oxidation. A similar pattern would be predicted starting with four uncoupled Mn^{2+}.

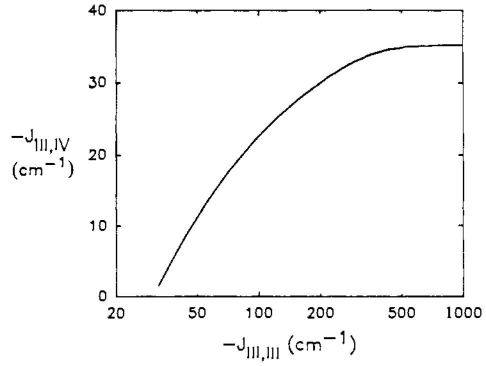

Figure 3. Calculated exchange interactions consistent with first-flash susceptibility data for a Mn dimer model for $S_1 \rightarrow S_2$ assuming Mn_2(1,II,III) → Mn_2(III,IV).

There are two mechanisms to consider that could produce the large increase in paramagnetism of the water-oxidizing complex observed for the $S_1 \rightarrow S_2$ transition. The most plausible is to decrease the electronic coupling between manganese ions within a chemically discrete cluster upon removal of an electron.

The other mechanism for changing the spin state of a coordination complex is by changing the electronic ground-state configuration of the individual ions, as was illustrated by the photodissociation of MbCO. However, the possibility of spin-state changes for the water-oxidizing complex is not supported by model compound studies. Mn^{2+} complexes rarely exhibit low-spin electronic states; none are known with protein or amino acid type ligands. The more applicable Mn^{3+} oxidation state has been found to form low-spin complexes, but this occurs with strong-field ligands such as porphyrins, which are not relevant ligands for manganese in the water-oxidizing complex. The situation with regard to Mn^{4+} is not as well documented. Also, spin-state changes have not been invoked to account for the EPR or the manganese UV and X-ray absorption changes accompanying S-state advancement.[2,3]

On the other hand, magnetic changes arising from differences in interion coupling are widely observed; some examples are given in Table I for dimanganese complexes. We will focus only on the first-flash change associated with the $S_1 \rightarrow S_2$ step and, for the sake of simplicity, initially consider an oversimplified dimer model in which only two of the four manganese ions are assumed coupled. This dimer model has proven excellent for predicting both the magnetic susceptibility and EPR properties of strongly coupled dimanganese complexes.[15] It assumes two Mn^{3+} ions in the S_1 state interacting by an isotropic Heisenberg exchange interaction, $J_{\text{III,III}}$, to produce a manifold of electronic states having total spin S = 4, 3, 2, 1, and 0. Upon oxidation a mixed-valence dimanganese(III,IV) species forms, having coupling $J_{\text{III,IV}}$ and producing states of total spin $S = {}^7/_2, {}^5/_2, {}^3/_2$, and ${}^1/_2$. With this model we can calculate what are the ranges of values of $J_{\text{III,III}}$ and $J_{\text{III,IV}}$ that could account for the large increase in the paramagnetism upon $S_1 \rightarrow S_2$ advancement. The results of this standard calculation are summarized in Figure 3. We know that $J_{\text{III,IV}}$ would have to be antiferromagnetic in sign in order to account for the formation of a multiline EPR signal associated with this spin $S = {}^1/_2$ state.[6,16] As an example, if we take a value

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(15) Cooper, S. R.; Dismukes, G. C.; Klein, M. P.; Calvin, M. C. *J. Am. Chem. Soc.* **1978**, *100*, 7248–7252.

(16) Dismukes, G. C.; Ferris, K.; Watnick, P. *Photobiochem. Photobiophys.* **1982**, *3*, 243–256.

of $J_{\mathrm{III,IV}} = -20$ cm^{-1}, which is within the range of estimates predicted for the S_2 state from the temperature dependence of the multiline EPR signal,[17,18] then Figure 3 predicts a value for $J_{\mathrm{III,III}} = -80$ cm^{-1}. That is, to account for the observed increase in paramagnetism, this dimer model predicts that there must be a *reduction* by 60 cm^{-1} in the magnitude of the electron-exchange interaction between the manganese ions upon oxidation to the mixed-valence S_2 state.

We now ask whether analogous changes in the susceptibility have been observed for dimanganese complexes undergoing the same oxidation change. The results from the literature for a number of single-μ_2-oxo- and di-μ_2-oxo-bridged dimanganese complexes having oxidation states $\mathrm{Mn_2(III,III)}$ and $\mathrm{Mn_2(III,IV)}$ are summarized in Table I. These molecules are particularly relevant because they possess oxo linkages that create Mn–Mn separations of 3.1 Å (single μ_2-oxo) and 2.7 Å (di-μ_2-oxo). These compare well with the photosynthetic site where two apparent Mn–Mn (or other third-row atom) distances have been proposed at 3.3 and 2.7 Å based upon analysis of manganese EXAFS data.[19] Here we see that, contrary to the results for the $S_1 \rightarrow S_2$ reaction, there have been found only large *decreases* in the paramagnetism upon oxidation to the mixed-valence state, attributable to a large *increase* in the antiferromagnetic coupling term. This is opposite to the case found for the $S_1 \rightarrow S_2$ conversion. We conclude that the large increase in paramagnetism observed for the photosynthetic manganese cluster for the $S_1 \rightarrow S_2$ reaction cannot be accounted for by the available data on dimanganese complexes. This means that the identity or structure of the bridging ligands in the enzyme must be different or that there are more than two coupled Mn ions responsible for the increase in magnetism. There is good evidence from EPR that in the S_2 state there is electronic coupling between at least 3 and possibly all 4 Mn ions within a chemically discrete cluster.[6,16,18] This electronic communication between the Mn ions is ultimately responsible for the multielectron catalytic properties of the water-oxidizing complex (eq 1).

In support of this interpretation magnetic changes have been reported for two tetramanganese complexes in oxidation states 4 Mn(III) and 3 Mn(III)Mn(IV), which agree closely with the observed $S_1 \rightarrow S_2$ increase in Figure 2.[20,21] The formulas for these structurally dissimilar complexes are $[\mathrm{Mn_4O_2(O_2CMe)_7(bpy)_2}]^{3+}$, which possesses the C_{2v}-symmetry butterfly core $\mathrm{Mn_4O_2}^{8+}$, and the anion $[\mathrm{Mn_4O_3Cl_6(HIm)(O_2CMe)_3}]^{2-}$, which possesses a C_{3v}-symmetry core comprised of a flattened trigonal pyramid $\mathrm{Mn_4O_3Cl}^{6+}$, respectively. The increase in the magnetic susceptibility reported for the anion vs the cation is 14.4 $\mu_B{}^2$ at 300 K.[29] While this pattern is revealing about possible oxidation state differences, it also includes magnetic changes arising from the different structures of these cores and the different ligand types employed. To our knowledge there are no other mixed-valent Mn tetramers in the oxidation state 3 Mn(III)Mn(IV) that have been characterized by magnetic susceptibility to broaden the examples for comparison with the S_2 state.

A large positive increase in magnetic susceptibility has recently been found arising from protonation of a μ_2-oxo bridge in the symmetrical μ_2-oxo adamantane core of $\mathrm{Mn_4O_6(TACN)_4}^{4+}$ to form a lower symmetry core containing a longer Mn–OH–Mn bond in $\mathrm{Mn_4O_5(OH)(TACN)_4}^{5+}$, emphasizing the sensitive dependence of the magnetic coupling on the bridging ligand structure.[30] Protonation state changes of the S states have also been measured in PSII membranes, although the site of deprotonation has not yet been identified. This has shown that no net proton release occurs for the $S_1 \rightarrow S_2$ oxidation reaction,[31] thus deemphasizing what possible contribution such changes might contribute to this susceptibility difference. The lack of net proton release does not discount the possibility that internal proton migration might occur and thereby affect the magnetism of the manganese cluster.

As a final comment, we note that the magnetic changes in Figure 2 are not cyclical, since the same base-line level of magnetism is not recovered after four flashes. This is partially attributable to the formation of net paramagnetism from O_2 production, mostly on flash 3. However, the net increase after four flashes is much greater than can be accounted for by this alone. The large increase seen on the first flash in samples initially dark adapted for 15 min or longer seems to be the source of this noncyclical behavior. A possible explanation may be that conversion occurs in the dark between an "active" S_1 state having a greater paramagnetism in samples that are turning over and a "resting" S_1 state having low paramagnetism in samples that are dark adapted for 15 min or longer. Conversion of this type has also been proposed to account for changes in the O_2 uptake rate in the S_1 state[22] and for changes in the EPR line shape and temperature dependence of the manganese site in the S_2 state in dark-adapted vs light-adapted samples.[22,23] Quantitative analysis to extract the susceptibility changes for each of the S states is in progress and holds promise for further insights into the mechanism of water oxidation.

Experimental Section

Isolation of O_2-evolving Photosystem II core complexes using the detergent octyl β-D-glucopranoside was performed by using the methods described by Ghanotakis et al.[14] These complexes typically contain 4 Mn/PSII, 70 Chl/PSII, and 6–10 Chla/Chlb, and evolve O_2 at rates 800–900 (μmol/mg Chl)/h. The Kok parameters that characterize the misses (α) and double hits (β) of these complexes were measured by using flash photolysis/EPR spectroscopy to follow the S_2 multiline signal yield.[32] These were found to be identical with those for the more commonly used but less well-resolved Triton-solubilized (BBY type) PSII membrane fragments ($\alpha = 10\%$, $\beta = 5\%$). Similarly, the initial ratio of $S_1/S_0 = 75\%$ was found, arising from 25% centers having donor D^+ in its reduced form. Very important for the present studies, we found no evidence from EPR that these samples contain partially inactivated water-oxidizing centers that would be capable of reaching S_2 or S_3 but could not evolve O_2.[32] Evidently, those centers that are inactive in O_2 evolution are also incapable of advancing to S_2 or beyond, probably indicating an all or nothing inactivation of the water-oxidizing complex, much like that seen with various inhibitors.[33] Dark adaptation of the samples was performed by incubating the complexes in the dark at 0 °C for 15 min or longer, a treatment that restores the ability to generate the

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[17] Hansson, O.; Andreasson, L. E.; Vanngard, T. In *Adv. Photosynth. Res., Proc. 6th Int. Cong. Photosynth.*; Sybesma, C., Ed.; Martinus-Nijhoff: The Hague, 1984; Vol. I, pp 307–311.

[18] de Paula, J. C.; Beck, W. F.; Brudvig, G. W. *J. Am. Chem. Soc.* **1986**, *108*, 4002–4009.

[19] Guiles, R. D.; Yaccandra, V.; McDermott, A.; Britt, R. D.; Dexheimer, S. L.; Sauer, K.; Klein, M. P. In *Prog. Photosynth. Res., Proc. 7th Int. Cong. Photosynth.*; Biggins, J., Ed.; Martinus-Nijhoff: Dordrecht, 1987; Vol. I, pp 561–564.

[20] Vincent, J. B.; Christmas, C.; Huffman, J. C.; Christou, G.; Chang, H. R.; Hendrickson, D. N. *Chem. Commun.* **1987**, 236–238.

[21] Bashkin, J. S.; Chang, H. R.; Streib, W. E.; Huffman, J. C.; Hendrickson, D. N.; Christou, G. *J. Am. Chem. Soc.* **1987**, *109*, 6502–6504.

[22] Beck, W. F.; de Paula, J. C.; Brudvig, G. W. *Biochemistry* **1985**, *26*, 3035–3043.

[23] Dismukes, G. C. In *Manganese in Metabolism and Enzyme Function*; Schram, V. L., Wedler, F. C., Academic Press: New York.

[24] Wieghardt, K.; Bossek, U.; Bonvoisin, J.; Beauvillian, P.; Girerd, J.-J.; Nuber, B.; Weiss, J.; Heize, J. *Angew. Chem.* **1986**, *98*, 1026–1027.

[25] Wieghardt, K.; Bossek, U.; Zsolnai, L.; Huttner, G.; Blondin, G.; Girerd, J.-J.; Babonneau, F. *J. Chem. Soc., Chem. Commun.* **1987**, 651–653.

[26] Sheats, J. E.; Czernuszewicz, R. S.; Dismukes, G. C.; Rheingold, A. L.; Petrouleas, V.; Stubbe, J.; Armstrong, W. H.; Beer, R. H.; Lippard, S. J. *J. Am. Chem. Soc.* **1987**, *109*, 1435–1444.

[27] Sheats, J. E.; UnniNair, B. C.; Petrouleas, V.; Artandi, S.; Czernuszewicz, R. S.; Dismukes, G. C. In *Prog. Photosynth. Res., Proc. 7th Int. Cong. Photosynth.* Biggins, J., Ed.; Martinus-Nijhoff: Dordrecht, 1987; Vol. I, pp 721–724.

[28] Menage, S.; Girerd, J. J.; Gleizes, A. *J. Chem. Soc., Chem. Commun.* **1988**, 431.

[29] Susceptibilities in the solid state of the bis(imidazolinium) salt of the anion and the tris(perchlorato) salt of the cation.[20,21]

[30] Hagen, K. S.; Westmoreland, T. D.; Scott, M. J.; Armstrong, W. H. *J. Am. Chem. Soc.* **1989**, *111*, 1907.

[31] (a) Velthuys, B. R. *FEBS Lett.* **1980**, *115*, 167–170. (b) Bowes, M. H.; Crofts, A. R. *Biochim. Biophys. Acta* **1981**, *636*, 464–472. (c) Forster, V.; Hong, Y.-Q.; Junge, W. *Biochim. Biophys. Acta* **1981**, *638*, 141–152. (d) Inoue, Y. *Biochim. Biophys. Acta* **1981**, *634*, 309–320. (e) Wille, B.; Lavergne, J. *Photobiochem. Photobiophys.* **1982**, *4*, 131–144.

[32] Sivaraja, M.; Dismukes, G. C. *Isr. J. Chem.*, in press.

[33] Sivaraja, M.; Hunziker, D.; Dismukes, G. C. *Biochim. Biophys. Acta* **1988**, *936*, 228–235.

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"19-line" form of the S_2 multiline signal in maximum yield upon subsequent illumination either by a single flash at 0 °C or by continuous illumination at 195 K.

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Registry No. O_2, 7782-44-7; Mn, 7439-96-5.

Mechanism of the Lanthanum Bromide Assisted Electrochemical Aldolization of α-Bromo Ketones

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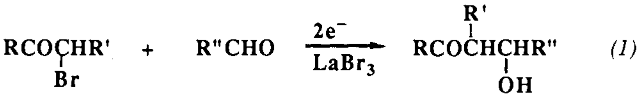
Abstract: Linear sweep voltammetry, preparative electrolyses under a variety of experimental conditions, and trapping experiments have been used to explore the mechanism of the formation of the aldol 2-benzoyl-1-phenylpropanol by electrochemical reduction of α-bromopropiophenone in the presence of benzaldehyde and lanthanum bromide. The aldol condensation occurs by reaction of the free (lithio) enolate with a lanthanum bromide–benzaldehyde–tetrahydrofuran complex. Electrochemical reduction of the bromo ketone forms the *Z* enolate highly stereospecifically. The erythro aldol is formed stereoselectively initially, but the condensation is reversible, and the equilibrium mixture of aldols, containing mostly the threo isomer, is isolated from the electrolysis.

We recently described the formation of β-hydroxy ketones (aldols) from the electrochemical reduction of α-bromo ketones (**1**) in the presence of aldehydes (**2**) and lanthanide salts (eq 1).[1]



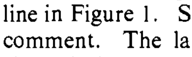
$$\mathrm{RCO\underset{Br}{C}HR'} + \mathrm{R''CHO} \xrightarrow[\mathrm{LaBr_3}]{2e^-} \mathrm{RCO\overset{R'}{C}H\underset{OH}{C}HR''} \qquad (1)$$

1 **2** **3**

Yields of aldol (**3**) were 0–1% in the absence of a lanthanide salt, but were up to 70% or more when such a salt was included in the reaction medium.[1] Relatively little is known concerning the effects of added metal ions on the electrochemical reduction of organic substances,[2,3] although one would expect that such effects ought to be substantial. This reaction represents a convenient system for investigating such effects: (a) the presence of the lanthanide salt is required for successful aldol formation; (b) the reduction potentials of the components of the electrode reaction, i.e., bromo ketones, aldehydes, and lanthanide salts are well separated (vide infra), removing any doubts concerning what the actual electroactive species might be; and (c) solutions of lanthanum halides at moderate concentrations in tetrahydrofuran are homogeneous, making the interpretation of voltammograms easier. We report herein the results of a study of the lanthanide-assisted aldolization of α-bromo ketones, using voltammetric methods, preparative electrolyses, and trapping experiments, which greatly clarifies the mechanism of the electrode reaction, including the complex role played by the metal ion.

Results

Voltammetry. Voltammetric measurements were carried out in tetrahydrofuran (THF) containing 0.5 M $LiClO_4$ (this relatively high supporting electrolyte concentration was employed to minimize uncompensated *iR* effects arising from the high electrical resistance of THF).[4] Linear potential sweep and cyclic voltammetric experiments were carried out at a freshly polished glassy carbon electrode at a sweep rate of 200 mV s^{-1}; potentials were measured relative to a Ag/0.1 M $AgNO_3$ reference electrode.[5] The compounds studied, alone and in various combinations, were α-bromopropiophenone (**4**), benzaldehyde (**5**), propiophenone (**6**),



$\mathrm{C_6H_5CO\underset{Br}{C}HCH_3}$

4

C_6H_5CHO

5

$C_6H_5COCH_2CH_3$

6

and anhydrous $LaBr_3$. The latter substance is insoluble in THF when initially added to the medium, but gradually dissolves as the mixture is stirred over a 15-min interval; a number of recent reports support the presumption that solubilization is associated with the complexation by $LaBr_3$ of at least three and possibly four molecules of THF.[6]

The voltammogram of bromo ketone **4** is shown as the solid line in Figure 1. Several features of this voltammogram require comment. The large peak at ca. −1.5 V corresponds to the electrolytic removal of the bromine atom, with concomitant conversion of **4** to the corresponding enolate (**7**).[7] There is a second, smaller, peak at ca. −2.1 V, close to the corresponding value for propiophenone (**6**) as determined in a separate experiment. More interestingly, there is a pronounced minimum in the voltammetric current at ca. −1.95 V. This is distinctly different behavior from that exhibited by a compound which is reduced in two consecutive steps with no intervening chemical reaction.[8] Instead, the voltammogram of **4** closely resembles that expected for a situation wherein the electroactive substance is reduced to

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[1] Fry, A. J.; Susla, M.; Weltz, M. *J. Org. Chem.* **1987**, *52*, 2496.

[2] (a) Sopher, D. W.; Utley, J. H. P. *J. Chem. Soc. Perkin Trans. 2* **1984**, 1361. (b) Fournier, F.; Berthelot, J.; Pascal, Y.-L. *Can. J. Chem.* **1983**, *61*, 2121.

[3] (a) There is a very large literature on metal ions as mediators of electrochemical behavior,[3b] but generally in such reactions the metal ion is the electroactive species; we are concerned in the present instance with reactions in which the electroactive species is an organic substrate whose electrochemical behavior is altered by the presence of the metal salt. (b) Torii, S. *Synthesis* **1986**, 873.

[4] Kadish, K. M.; Ding, J. Q.; Malinski, t. *Anal. Chem.* **1984**, *56*, 1741.

[5] The potential of this electrode is +0.35 V versus SCE.

[6] (a) Hazin, P. N.; Huffman, J. C.; Bruno, J. W. *Organometallics* **1987**, *6*, 23. (b) Wengi, C.; Zhongsheng, J.; Yan, X.; Yuguo, F.; Guangdi, Y. *Inorg. Chim. Acta* **1987**, *130*, 125. (c) Brittain, H. G.; Wayda, A. L. *Inorg. Chim. Acta* **1984**, *95*, 21.

[7] Fry, A. J. *Synthetic Organic Electrochemistry*, 2nd ed.; Wiley: New York, in press, Chapter 5.

[8] Demortier, A.; Bard, A. J. *J. Am. Chem. Soc.* **1973**, *95*, 3495.

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