Simulation of the "$S_0$-state" EPR signal from the Mn cluster in photosystem II. Evidence for a piece of a thermally accessible O₂-binding state.

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Introduction

Cyanobacteria, algae and higher plants inherit photosystem II (PSII) which light-dependently extracts every four electrons from a water dimer to emit four protons and evolve an O₂ molecule on the donor side of PSII reaction center, P680. A phenomenological reaction scheme was found to involve five intermediates, called Kok's S-states, as depicted in Fig.1. A tetranuclear Mn cluster catalyzes this water oxidation reaction together with cofactor ions, Ca²⁺ and Cl⁻. The molecular structure of PSII including this Mn cluster was very recently unveiled with use of the PSII crystal from *S. elongatus* at 3.8 Å resolution (A. Zouni et al). The position of the Mn cluster in PSII D1 polypeptide and a rough electron density cloud attributable to the Mn cluster were elucidated, although the cluster structure is still largely unknown.

So far, the EPR spectroscopy has been used to provide valuable information not only on the magnetic states of the Mn clusters in various redox states but also the molecular structure behind the observed EPR signals. The first discovered multiline signal (MLS) from the S₂-state has been most extensively studied. Recently, two groups (Messinger et al.'1997,'1998; Åhlring et al.'1997,'1998) have independently detected a similar MLS from the S₀-state PSII membranes added 1-3 % methanol. This S₀-MLS is much more complicated than the S₂-MLS. In this paper, we will present the first reasonable simulation of this new signal, by decomposing it into a main signal due to the S₀-state Mn cluster, ~270G-wide signal due to the thermally populated S₄-state Mn cluster, which loosely binds O₂, and widely extended irregular structures. The structural implication of these components will be discussed.

Theoretical Consideration

The magnetic state for the S₀-state Mn cluster at helium temperatures was experimentally found to be an isolated ground doublet ($S=1/2$) that should be described by

$$H_{S(0)}=\mu_B S \cdot g \cdot B + \sum_{i=a-d} S \cdot A_i \cdot I_i$$

$$g_i = \sum_{i=a-d} P_i g_i$$

$$A_i = P_i a_i$$

where $\mu_B$ is the Bohr magneton, $I_i$ is a nuclear spin operator of the $i$th Mn nucleus, $g_i$ is the intrinsic g-tensor of the $i$th Mn ion ($i=a-d$), $a_i$ is its intrinsic hyperfine tensor, and $P_i$ is the projection factor of the $i$th Mn spin $S_i$ against the total spin $S$, which can be calculated from the $S=1/2$ ground state wave function for the spin-exchange Hamiltonian $H_{ex} = \sum_{i<j} S_i \cdot S_j$ (see e.g. Hasegawa et al.). Here, we are concerned with the EPR signals from powder samples. Then, a molecular coordinate system may be arbitrarily chosen and
here the principal coordinate system of the tensor $g_a$ has been chosen as reference. We have also assumed that Mn$^\text{II}$, Mn$^\text{III}$ and/or Mn$^\text{IV}$ ions in the cluster would be in each octahedral ligand field, obeying Hund’s rule. Then, Mn$^\text{II}$ ion must have a zero LS-coupling to cause a free-electron-like $g$-tensor ($g^\text{III}=g_e=2.0023$) and an isotropic $a$-tensor ($a^\text{III}=a_0$), while Mn$^\text{IV}$ ion exhibits the largest LS-coupling as given by

$$\lambda_{\text{IV}} = \lambda_{\text{III}} b_{\text{III}}$$

with $\lambda_{\text{IV}}$ taking the atomic value ($\approx 134$ cm$^{-1}$) and $b_{\text{III}}$ representing a reduction factor from free to binding state. This yields near-isotropic $g^\text{III}$ - and $a^\text{III}$-tensors: $g^\text{III}=g_e-8\lambda_{\text{III}} b_{\text{III}}/\Delta_{\text{III}}$, and $a^\text{III}=a_0(\kappa+g_e g^\text{III})$, where $\Delta_{\text{III}}$ is the energy gap between $\gamma$ and $\delta$ orbitals. But, the Jahn-Teller ion Mn$^\text{III}$ must show a remarkable anisotropy in both the $g^\text{III}$ and $a^\text{III}$-tensors, in spite of the LS-coupling $\lambda_{\text{III}} = \lambda_{\text{III}} b_{\text{III}}$ being intermediate, where $\lambda_{\text{III}}$ is an atomic value and $b_{\text{III}}$ represents the effect of binding to the ligand site. Let us remember that the $d$-hole wave function of this ion can be described by

$$\Psi^\text{III}(\delta) = \cos(\delta) x^2 - y^2 + \sin(\delta) z^2 - r^2. \quad (4)$$

Then, the principal values of these tensors can be conveniently parameterized as (Abraham and Price)

$$g^\text{III}_x = g_e - (8\lambda_{\text{III}} b_{\text{III}}/\Delta_{\text{III}}) \cos^2(\delta + \pi/3),$$
$$g^\text{III}_y = g_e - (8\lambda_{\text{III}} b_{\text{III}}/\Delta_{\text{III}}) \cos^2(\delta - \pi/3),$$
$$g^\text{III}_z = g_e - (8\lambda_{\text{III}} b_{\text{III}}/\Delta_{\text{III}}) \cos^2(\delta), \quad (5)$$
$$a^\text{III}_x = a_0 \{ \kappa + g_e g^\text{III} + \cos(2\delta + \pi/3)/7 \},$$
$$a^\text{III}_y = a_0 \{ \kappa + g_e g^\text{III} + \cos(2\delta - \pi/3)/7 \},$$
$$a^\text{III}_z = a_0 \{ \kappa + g_e g^\text{III} - \cos(2\delta)/7 \}, \quad (6)$$

which are notably described by only four parameters, $\delta$, $b$, $a_0$, and $\kappa$, since $\lambda_{\text{III}}$ and $\Delta_{\text{III}}$ may be in general fixed at the standard values (aqou Mn$^\text{III}$ ion), 88 cm$^{-1}$ and 2100 cm$^{-1}$, respectively. For simplicity and as a reasonable approximation, the $g$-tensor of the $i$th Mn ion has been assumed to be collinear to the hyperfine tensor $a_i$. An extreme case of $\delta=0^\circ$ represents a usual Jahn-Teller distortion to make a $d(x^2-y^2)$-hole, yielding the axial tensors satisfying the relations: $g_x = g_y = g_e$ and (usually) $|a_{\gamma}| > |a_{\delta}|$, although the so-called “opposite Jahn-Teller distortion” with a $d(3z^2-r^2)$-hole can also occur at $\delta=90^\circ$ accompanied with unusual relations for axial tensors: $g_x = g_y = g_e$ and $|a_{\delta}| > |a_{\gamma}|$. Similarly, both the usual and the opposite Jahn-Teller distortions with the rhombic symmetry can take place at $\delta=15^\circ$ and $\delta=75^\circ$, satisfying $(g_x < g_y < g_{\delta}, |a_{\delta}| < |a_{\gamma}|)$ and $(g_x < g_y < g_{\gamma}, |a_{\gamma}| < |a_{\delta}|)$, respectively.

The effective $g$-tensor and the effective hyperfine tensors $\{A_i\}$ may strongly reflect the structure of the Mn cluster via the characteristic projection factors $\{P_i\}$ which may significantly change as some spin-exchange interactions are modified. The S$_0$-state multiline signal provided us an interesting example that could be detected by Messinger et al. (1997, 1998) and Åhlring et al. (1997, 1998) by adding a few percent of methanol to PSII from spinach and later by Boussac et al. (1999) without adding methanol in PSII from S. elongatus. By careful inspection of the reported spectra, we picked up three important observations: (a) the spectrum is more than 2500 G wide in total, but it appears to consist of 24-26 well resolved multilines in a central 2000-2200G region, called “main component”, and rather irregular features with a different average spacing outside it, called “extended component”; (b) the central $g$-value is appreciably below $g=2.0$; (c) a central region of the spectrum may contain a small “$\sim$270G-wide signal” to cause a clear deviation from a single Gaussian derivative spectrum (Åhlring et al. 1998; Boussac et al. 2000), although this third component is not observed by Messinger et al. (1998) who added methanol, FCCP and PPBQ. We speculate that this $\sim$270G-wide signal might have appeared only in some S$_0$-state sample added 1-3 % methanol and/or something else, but not by illumination of one Xe flash on the reduced “S$_{-1}$-state” sample. Namely, we would hypothesize that this narrow signal might have arisen from a small amount of thermally populated S$_4$-state Mn clusters at around 0 °C which would require a free energy difference of order 1.4 kcal/mol between S$_4$ (an O$_2$-binding state) and S$_0$ (a H$_2$O-binding state). If this is the case, the triplet O$_2$ molecule must be loosely bound to the doublet S$_0$-Mn$_4$ cluster to cause a doublet bound state and a quartet bound state, both of which may be thermally populated even at helium temperatures. Indeed, this S=1/2 S$_4$ cluster can exhibit the observed EPR width of $\sim$270G, because the spin Hamiltonian to describe this EPR signal is given by

$$H_{\text{S}(4)}=\mu_B S \cdot g_S \cdot B + \sum_{i=a-d} S \cdot (-A_i/3) \cdot I_i, \quad (7)$$

where $g_S=g/3+4g_0/3$ with $g_0$ being a $g$-tensor of the O$_2$ spin. Note that four effective hyperfine tensors are scaled by a factor, $-1/3$, yielding a reduced width, $\sim$750 G/3$\sim$250 G, of the EPR signal in agreement with the observation. This coupling state requires that an effective spin-exchange coupling between O$_2$ and Mn$_4$ spins must be much smaller than an excited state energy (30 cm$^{-1}$, Åhlring et al’1998) of the S$_0$-state Mn cluster. Such a weak coupling can be either antiferromagnetic or ferromagnetic. If it is antiferromagnetic, the doublet will become the ground state to give rise to a prominent $\sim$270 G wide EPR
signal as observed by Åhlring et al. ’1998 and Boussac et al. ’2000, and then the quartet will become an excited state to cause a weak and very wide spectrum, which may be still detectable as fine structures around g=2.0 if the zero-field splitting (ZFS) tensor happens to become small. On the other hand, if it is ferromagnetic, the two multiplets will exchange the standing positions, possibly causing an opposite situation as observed by Messinger et al. ’1998. This quartet O2-binding S4-state may be one of the possible mechanisms for the widely extending irregular features, although an inhomogeneous distribution of the S0-state Mn clusters is more likely. Anyway, we will discuss no longer about this extended component.

In XANES experiments on the S0-state in PSII membranes illuminated three Xe flashes, two conflicting conclusions on the valence assignment for four Mn spins were derived, Mn(III,III,III,IV) (Kusunoki et al. ’1993, Iussolino et al. ’1998) vs Mn(II,III,IV,IV) (Roelofs et al. ’1996), as illustrated in Fig. 1. The latter assignment was proposed along with a “dimmer-of-dimers” model for the organization of four Mn ions, under an implicit assumption that this might block an electron transfer between the Mn(II,III) and the Mn(IV,IV) dimeric subunits, although a recently reported X-ray structure (A. Zouni et al) seems to be inconsistent with this assumption. Furthermore, by this model including two parallel strong-antiferromagnetic exchange interactions, it would be difficult to understand why resolvable multiline can appear in the presence of a few % of methanol. On the other hand, the former assignment is consistent with any model that allows delocalization of d-holes in the cluster for positive charge accumulation. Therefore, we have thoroughly examined on the Mn(III,III,III,IV) oxidation state.

Results and Discussions

In Fig. 2, we show the experimental S0 multiline signal (spectrum a, Åhlring et al. ’1998) to be compared with one of our best simulations (spectrum b, solid line) which was found to be a superposition of doublet spectra, b-1 (dashed line) and b-2 (dashed-dotted line) due to 92.2 % S0-Mn(III,III,III,IV) clusters and 7.8 % S4-Mn(III,III,III,IV)-O2 clusters, respectively. Significantly, the theoretical spectrum b-1 satisfactorily reproduces spectrum a with respect to all the 26 resolved hyperfine structures as well as a whole spectral shape, except for a systematic deviation as clearly seen in the region 3100-3600G. As mentioned above, this systematic deviation can be nicely compensated by a weak ~270 G-wide Gaussian EPR signal indicative of a thermally populated S0-Mn cluster at 0 °C containing a weak-antiferromagnetic couple of the S0-Mn(III,III,III,IV) cluster and O2 molecule.

In Table 1, we show the values of all the EPR parameters which give rise to this best simulation and hence characterize the S0-Mn(III,III,III,IV) cluster. First of all, it should be noted that the effective g-tensor is near-axial, the principal values, \( g_x=1.9557 \) and \( g_y=1.9578 \), are relatively small but \( g_z=2.0530 \) is relatively largely. This brings an asymmetric spectrum around \( g=2.0 \), in agreement with our observation (b).

Significantly, this unusual near-axial g-tensor can be reasonably explained in terms of the "diamond

| Table 1 EPR parameters for the S0-state Mn cluster, derived from simulation of the multiline signalc |
|-----------------------------------|---|---|---|---|
| \( i \): Mn(valence) | a: Mn(III) | b: Mn(III) | c: Mn(III) | d: Mn(IV) |
| \( \delta \) | 13° | 87° | 87° | 87° |
| \( b_i \) | 1.0 | 1.0 | 1.0 | 0.6 |
| \( a_{0} \) | -72 G | -73 G | -70 G | -77 G |
| \( a_{0}^\kappa \) | -73 G | -74 G | -71 G | -78 G |
| \( P_i \) | -1.0093 | 1.1785 | 1.7167 | -0.8860 |
| \( g_{0i} \), \( g_{1i} \) | 1.9994,1.9867,1.9705 | 1.9787,1.9757,2.0022 | 1.9787,1.9757,2.0022 | 1.9809,1.9809,1.9809 |
| \( a_{0}, a_{1}, a_{2} \) (G) | -72.9,-81.8,-65.0 | -68.5,-70.7,-83.5 | -65.7,-67.8,-80.1 | -78.7,-78.7,-78.7 |
| \( \alpha_i, \beta_i, \gamma_i \) | 0.0°, 0.0°, 0.0° | 12.3°, -9.7°, 2.0° | -7.2°, 7.5°, 2.0° | - |
aThese values correspond to a diamond-type Mn₄ cluster with J_ab=200, J_ac=70, J_ad=20, J_bc=5, J_cd=0, and J_ded=40, in units of cm⁻¹. bThe effective g-tensor is near-axial with principal values:1.9557, 1.9598, 2.0530. cThe inhomogeneous broadening of each hyperfine absorption line was found to be δB=25×(0.006B²)¹/².

structure” model illustrated in Fig. 3. In this S₀ Mn(III,III,III;IV) cluster, the Mn₄ cluster is ligated by two perpendicular µ₂-oxo-µ₁-oxo bridges with different bridge lengths (R_ab=2.7Å, R_bc=2.75-2.8Å), indicating that the Jahn-Teller distortion must be near-rhombic and become a usual case when the principal z-axis is chosen along the Mn₃ → µ₁-oxo bond, in agreement with the phase angle δ_b=13°. Furthermore, both Mn₃ and Mn₄ ions are similarly ligated by only one µ₂-oxo bridge along the z-axis, indicating that both of their d-hole wave functions must be the opposite Jahn-Teller case with near-axial symmetry, in agreement with the phase angles, δ_b=87° and δ_c=87°. In this manganese trimer, only the exchange interaction J_{ab} is expected to be strong-antiferromagnetic (> 180 cm⁻¹), J_{bc} to be intermediate in the range 40-100 cm⁻¹, and J_{bc} to be weak antiferro- or ferro-magnetic, because of such EXAFS-derived interatomic distances as indicated. This trimeric core can cause such spin-projection factors as yielding the resolvable hyperfine structures in the EPR signal, sensitively depending upon the OEC structure. The observed unusual axial g-tensor with low g_x and g_y values is now understandable as a result of the interesting situation that both unusual near-axial Jahn-Teller ions, Mn₃ and Mn₄, take a large positive projection factor, and a usual near-rhombic Jahn-Teller ion, Mn₄, cooperatively takes a large negative value. A typical set of {P_i} are given in Table 1, although they have not been thoroughly optimized because of their correlation with the parameters, b, a₀ and a₀κ.

The miner component in the S₀ MLS called ~270G-wide EPR signal has been attributed to the S₄ state, although this S₄ has been so far speculated as transient and very unstable because no spectroscopic evidence has been presented. However, if S₄ is defined as the final intermediate state in the course of spontaneous decay from S₃ to S₀, the Mn₄ cluster in S₄ must have a similar structure and a similar electronic state to S₀, expect that a synthesized O₂ molecule is still bound near the Mn₄ cluster. Therefore, it would be reasonable to assume that an energy difference between S₀ and S₄ might be of order 1.4-2 kcal/mol. Experimental proof for this important prediction is desired.

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References