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# Simulation of the "S<sub>0</sub>-state" EPR signal from the Mn cluster in photosystem II. Evidence for a piece of a thermally accessible O<sub>2</sub>-binding state.

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### Introduction

algae and higher plants Cyanobacteria, inherit photosystem II (PSII) which light-dependently extracts every four electrons from a water dimer to emit four protons and evolve an O<sub>2</sub> 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,  $Ca^{2+}$  and  $C1^{-}$ . 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  $S_2$ -state



*Fig.1* Phenomenological Kok's S-state scheme.  $S_1$  is stable and  $S_4$  spontaneously decays into  $S_0$  after releasing  $O_2$ .in exchange with  $H_2O$ . A straight solid arrow represents abstraction of an electron by  $P680^+$ , the proton release pattern is not established, and the proposed valence states on  $S_0$ -Mn<sub>4</sub> are indicated.  $S_{-1}$  is generated by two-electron reduction from  $S_1$ .

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 S<sub>0</sub>-state PSII membranes added 1-3 % methanol. This S<sub>0</sub>-MLS is much more complicated than the S<sub>2</sub>-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 S<sub>0</sub>-state Mn cluster, ~270G-wide signal due to the thermally populated S<sub>4</sub>-state Mn cluster, which loosely binds O<sub>2</sub>, and widely extended irregular structures. The structural implication of these components will be discussed.

#### **Theoretical Consideration**

The magnetic state for the  $S_0$ -state Mn cluster at helium temperatures was experimentally found to be an isolated ground doublet (S=1/2) that should be described by

$$H_{S(0)} = \mu_{\rm B} \boldsymbol{S} \boldsymbol{\bullet} \boldsymbol{g} \boldsymbol{\bullet} \boldsymbol{B} + \sum_{i=\text{a-d}} \boldsymbol{S} \boldsymbol{\bullet} \boldsymbol{A}_i \boldsymbol{\bullet} \boldsymbol{I}_i \tag{1}$$

$$\boldsymbol{g} = \sum_{i=a-d} P_i \boldsymbol{g}_i \tag{2}$$

$$A_i = P_i a_i \tag{3}$$

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 gtensor 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<sup>II</sup>, Mn<sup>III</sup> and/or Mn<sup>IV</sup> ions in the cluster would be in each octahedral ligand field, obeying Hund's rule. Then, Mn<sup>II</sup> ion must have a zero LS-coupling to cause a free-electron-like *g*-tensor ( $g^{II}=g_e=2.0023$ ) and an isotropic *a*-tensor ( $a^{II}=a_0\kappa$ ), while Mn<sup>IV</sup> ion exhibits the largest LS-coupling as given by  $\lambda_{IV} = \lambda_{IV}b_{IV}$  with  $\lambda_{IV}$  taking the atomic value ( $\approx 134 \text{ cm}^{-1}$ ) and  $b_{IV}$  representing a reduction factor from free to binding state. This yields near-isotropic  $g^{IV}$ - and  $a^{IV}$ -tensors:  $g^{IV} = g_e - 8\lambda_{IV}b_{IV}/\Delta_{IV}$ , and  $a^{IV} = a_0(\kappa + g_e - g^{IV})$ , where  $\Delta_{IV}$  is the energy gap between d $\gamma$  and de orbitals. But, the Jahn-Teller ion Mn<sup>III</sup> must show a remarkable anisotropy in both the  $g^{III}$ - and  $a^{III}$ -tensors, in spite of the LS-coupling  $\lambda_{III} = \lambda_{III}b_{III}$  being intermediate, where  $\lambda_{III}$  is an atomic value and  $b_{III}$  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

$$\Psi^{\text{III}}(\delta) = \cos(\delta) |x^2 - y^2\rangle + \sin(\delta) |3z^2 - r^2\rangle.$$
(4)

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

$$g_{x}^{III} = g_{e} - (8\lambda_{III}b/\Delta_{III})\cos^{2}(\delta + \pi/3), \quad \text{and} \quad a_{x}^{III} = a_{0} \{\kappa + g_{e} - g_{x}^{III} + \cos(2\delta + \pi/3)/7\}, \\ g_{y}^{III} = g_{e} - (8\lambda_{III}b/\Delta_{III})\cos^{2}(\delta - \pi/3), \quad a_{y}^{III} = a_{0} \{\kappa + g_{e} - g_{y}^{III} + \cos(2\delta - \pi/3)/7\}, \\ g_{z}^{III} = g_{e} - (8\lambda_{III}b/\Delta_{III})\cos^{2}(\delta), \quad (5) \quad a_{z}^{III} = a_{0} \{\kappa + g_{e} - g_{z}^{III} + \cos(2\delta - \pi/3)/7\}, \\ g_{z}^{III} = a_{0} \{\kappa + g_{e} - g_{z}^{III} - \cos(2\delta)/7\}, \quad (6)$$

which are notably described by only four parameters,  $\delta$ , b,  $a_0$  and  $\kappa$ , since  $\lambda_{III}$  and  $\Delta_{III}$  may be in general fixed at the standard values (aquo Mn<sup>III</sup> ion), 88 cm<sup>-1</sup> and 2100 cm<sup>-1</sup>, respectively. For simplicity and as a reasonable approximation, the  $g_i$ -tensor of the *i*th Mn ion has been assumed to be collinear to the hyperfine tensor  $a_i$  (*i*=a-d). 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:  $g_x = g_y > g_z$  and (usually)  $|a_x| = |a_y| > |a_z|$ , 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:  $g_x = g_y < g_z = g_e$  and  $|a_x| = |a_y| < |a_z|$ . 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 ( $g_z < g_y < g_z$ ,  $|a_z| < |a_y| > |a_y|$ ) and ( $g_y < g_x < g_z$ ,  $|a_y| < |a_z|$ ), respectively.

 $\delta$ =75°, satisfying ( $g_z < g_y < g_x$ ,  $|a_z| < |a_x| < |a_y|$ ) and ( $g_y < g_x < g_z$ ,  $|a_x| < |a_y| < |a_z|$ ), respectively. The effective *g*-tensor and the effective hyperfine tensors {*A*<sub>i</sub>} may strongly reflect the structure of the Mn cluster via the characteristic projection factors  $\{P_i\}$  which may significantly change as some spinexchange interactions are modified. The S<sub>0</sub>-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 "~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 ~270G-wide signal might have appeared only in some S<sub>0</sub>-state sample that was generated by illumination of three Xe flashes on the 100 % S<sub>1</sub>-state sample added 1-3 % methanol and/or something else, but not by illumination of one Xe flash on the reduced "S<sub>-1</sub>-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  $H_2O$ -binding state). If this is the case, the triplet  $O_2$  molecule must be loosely bound to the doublet S<sub>0</sub>-Mn<sub>4</sub> 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<sub>4</sub> cluster can exhibit the observed EPR width of ~270G, because the spin Hamiltonian to describe this EPR signal is given by

$$H_{S(4)} = \mu_{\mathbf{B}} \mathbf{S} \bullet \mathbf{g}_{(4)} \bullet \mathbf{B} + \sum_{i=a-d} \mathbf{S} \bullet (-\mathbf{A}_{i}/3) \bullet \mathbf{I}_{i}, \qquad (7)$$

where  $g_{(4)}=-g/3+4g_{O2}/3$  with  $g_{O2}$  being a *g*-tensor of the O<sub>2</sub> spin. Note that four effective hyperfine tensors are scaled by a factor, -1/3, yielding a reduced width, ~750 G/3=~250 G, of the EPR signal in agreement with the observation. This coupling state requires that an effective spin-exchange coupling between O<sub>2</sub> and Mn<sub>4</sub> spins must be much smaller than an excited state energy (~30 cm<sup>-1</sup>, Åhlring et al'1998) of the S<sub>0</sub>-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 ~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 O<sub>2</sub>-binding S<sub>4</sub>-state may be one of the possible mechanisms for the widely extending irregular features, although an inhomogeneous distribution of the S<sub>0</sub>-state Mn clusters is more likely. Anyway, we will discuss no longer about this extended component.

In XANES experiments on the  $S_0$ -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 multilines 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  $S_0$  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 (dashdotted line) due to 92.2 % S<sub>0</sub>-Mn(III,III,III,IV) clusters and 7.8 % S<sub>4</sub>-Mn(III,III,III,IV)-O<sub>2</sub> 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 S<sub>4</sub> Mn cluster at 0 °C containing a weakantiferromagnetic couple of the S<sub>0</sub>-Mn(III,III,III,IV) cluster and O<sub>2</sub> molecule.

In Table 1, we show the values of all the EPR parameters which give rise to this best simulation and hence characterize the  $S_0$ -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



*Fig.2* High-resolution  $S_0$  state EPR spectrum **a** (Åhlring et al'1998) and our simulation spectrum **b**, decomposed into two components, **b-1** and **b-2**, attributable to 92.2 % *S*=1/2 Mn(III,III,III,IV) and 7.8 % *S*=1/2 O<sub>2</sub>-Mn(III,III,IV) clusters. Extended irregular structures in whole region would be mostly due to the inhomogeneous distribution of the methanol-treated S<sub>0</sub>-state Mn clusters.

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"

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<i>i</i> : Mn(valence)	a: Mn(III)	b: Mn(III)	c: Mn(III)	d: Mn(IV)
$\delta_i$	13°	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
$P_i^{a}$	-1.0093	1.1785	1.7167	-0.8860
$g_{ix}, g_{iy}, g_{iz}^{b}$	1.9994,1.9867,1.9705	1.9787,1.9757,2.0022	1.9787,1.9757,2.0022	1.9809,1.9809,1.9809
$a_{ix}, a_{iy}, a_{iz}$ (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°	

Table 1 EPR parameters for the S<sub>0</sub>-state Mn cluster, derived from simulation of the multiline signal<sup>e</sup>

<sup>a</sup>These values correspond to a diamond-type Mn<sub>4</sub> cluster with  $J_{ab}=200$ ,  $J_{ac}=70$ ,  $J_{ad}=20$ ,  $J_{bc}=5$ ,  $J_{bd}=0$ , and  $J_{cd}=40$ , in units of cm<sup>-</sup> <sup>1</sup>. <sup>b</sup>The effective *g*-tensor is near-axial with principal values:1.9557, 1.9598, 2.0530. <sup>c</sup>The inhomogeneous broadening of each hyperfine absorption line was found to be  $\delta B = \{25+(0.006B)^2\}^{1/2}$ .

structure" model illustrated in *Fig. 3*. In this  $S_0$  Mn(III,III,III,IV) cluster, the Mn<sub>a</sub><sup>ff</sup> ion is ligated by two perpendicular  $\mu_2$ -oxo- $\mu_3$ -oxo bridges with different bridge lengths ( $R_{ab}\approx 2.7$ Å,  $R_{ac}\approx 2.75$ -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  $Mn_a^{III} \rightarrow \mu_3$ -oxo bond, in agreement with the in and phase angle  $\delta_a \approx 13^\circ$ . Furthermore, both Mn<sub>b</sub><sup>II</sup> <sup>11</sup> ions are similarly ligated by only one  $\mu_2$ -Mn<sub>c</sub><sup>m</sup> oxo bridge along the z-axis, indicating that both

of their d-hole wave functions must be the Jahn-Teller case opposite with near-axial symmetry, in agreement with the phase angles,  $\delta_b \approx 87^\circ$  and  $\delta_c \approx 87^\circ$ . In this manganese trimer, only the exchange interaction  $J_{ab}$  is expected to be strong-antiferromagnetic (> 180 cm<sup>-1</sup>),  $J_{ac}$  to be intermediate in the range 40-100 cm<sup>-1</sup>, 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 spinprojection



Fig.3 "Diamond-structure" model for the tetranuclear Mn cluster in OEC and the valence assignment in S<sub>0</sub>- and S<sub>4</sub>-states. order stabilize triangular In to the core  $[Mn_{a}^{III}Mn_{b}^{III}Mn_{c}^{III}O_{3}](R_{ab}\approx 2.7\text{\AA},$  $R_{\rm bc} \approx 3.2$ -*R*<sub>ac</sub>≈2.75-2.8Å, 3.3Å.) and to reconcile with the 3.8Å resolution X-ray structure (A. Zouni et al), we put a  $Ca^{2+}$  ion at the position as indicated. It is assumed that  $Mn_d^{IV}$  ion is mono- $\mu_2$ -oxo bridged to  $Mn_a^{III}$ , two H<sub>2</sub>O molecules are bound to  $Mn_b^{III}$  and  $Mn_c^{III}$  in  $S_0$ , and  $O_2$  is bound to either site of water binding in  $S_4$ .

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,  $Mn_b^{III}$  and  $Mn_c^{III}$ , take a large positive projection factor, and a usual near-rhombic Jahn-Teller ion,  $Mn_a^{III}$ , 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,  $a_0$  and  $a_0\kappa$ .

The miner component in the S<sub>0</sub> MLS called ~270G-wide EPR signal has been attributed to the S<sub>4</sub> state, although this S<sub>4</sub> has been so far speculated as transient and very unstable because no spectroscopic evidence has been presented. However, if S<sub>4</sub> is defined as the final intermediate state in the course of spontaneous decay from  $S_3$  to  $S_0$ , the  $Mn_4$  cluster in  $S_4$  must have a similar structure and a similar electronic state to  $S_0$ , except that a synthesized  $O_2$  molecule is still bound near the  $Mn_4$  cluster. Therefore, it would be reasonable to assume that an energy difference between S<sub>0</sub> and S<sub>4</sub> might be of order 1.4-2 kcal/mol. Experimental proof for this important prediction is desired.

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