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An interpretation of the peculiar magnetic properties of center X in Photosystem I in terms of a 2Fe-2S cluster

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The temperature dependence of the spin-lattice relaxation time of center X in the Photosystem I has been deduced from the relaxation broadening of the EPR line which appears when the temperature is higher than 10 K. The results indicate the presence of an excited level of energy 50 cm^{-1}. We then show that the remarkable properties of center X are well explained in terms of a 2Fe-2S cluster characterized by weak antiferromagnetic exchange interactions.

In Photosystem I (PS I) of algae and higher plants, light energy is used to transfer electrons through the thylakoid membrane via a pigment (P-700) and a chain of redox centers (A$_0$, A$_1$, X, A, B). Although the exact nature of P-700, A$_0$ and A$_1$ is not firmly established (see Ref. 1 for a review), it is now admitted that X, A and B are low-potential iron-sulfur centers [2]. On the basis of EPR [3] and Mossbauer [4] experiments, A and B are considered as two weakly interacting 4Fe-4S clusters, and there is good evidence for their location in a small polypeptide of about 8 kDa [5,6]. The nature of X is more controversial: a Mossbauer study has concluded that all the iron-sulfur centers of PS I are 4Fe-4S clusters [4], but recent studies indicate that X could be a 2Fe-2S cluster [7,8]. Due to their weak accuracy, iron content measurements which give 10–14 Fe by P-700 [9,10] cannot be used to determine the nature of X.

The properties of center X appear to be very

remarkable by comparison with usual iron-sulfur centers: (i) a very low redox potential of about −700 mV [11]; (ii) an EPR signal in the reduced state characterized by a very anisotropic $\tilde{g}$ tensor (2.08, 1.88, 1.78) and very broad lines; (iii) a very fast spin-lattice relaxation rate which leads to the disappearance of the EPR signal around 30 K [12].

We have measured the temperature dependence of the spin-lattice relaxation time T_1 of center X between 10 K and 21 K. The results indicate the presence of an excited level of energy 50 cm^{-1}. On that basis, a model is presented to explain the remarkable properties of center X. This work was presented in part at the 3rd I.C.B.I.C. Conference [13].

Spinach PS I particles were prepared as previously described [5]. The ratio Chl/P-700 was equal to 65, and the final concentration of chlorophyll in the EPR sample was about 10 mg/ml. The reduction was achieved by freezing under illumination in the presence of a 20 mM solution of sodium dithionite at pH 10. EPR spectra were recorded on a Varian E 112 spectrometer equipped with an Air-Products gaz-flow system. The tem-

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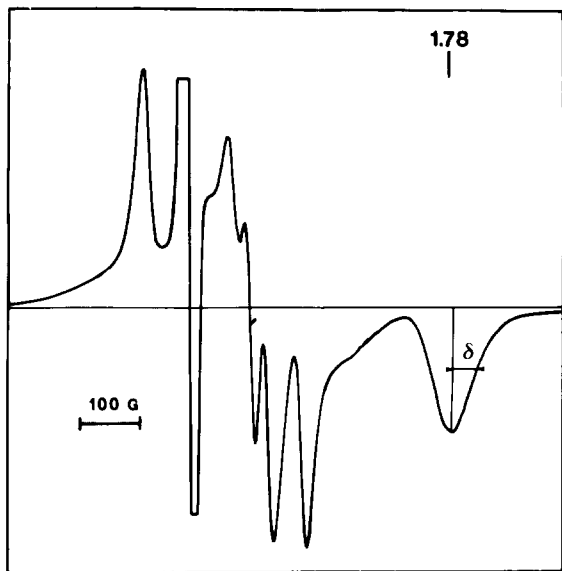
Fig. 1. EPR spectrum of reduced PS I particles. Experimental conditions: temperature, 7 K; microwave power, 10 mW; microwave frequency, 9290 MHz.

perature was measured before and after each spectrum with a calibrated thermocouple (Chromel VS Au-0.07% Fe) placed in an EPR tube partially filled with water.

In our preparation, the low-temperature EPR spectrum contains contributions from A, B and X (Fig. 1), and we have studied the X signal in the high-field region where A and B do not contribute. In the course of the study, we have observed that the shape of the spectrum was dependent on the orientation of the EPR tube relative to the field. This effect indicates that the particles are partially oriented in the sample, and is probably related to the high concentration used in the experiments. We have then selected the orientation corresponding to the maximum amplitude of the $g = 1.78$ peak. A detailed study of the temperature dependence of the EPR signal gives the following results.

(i) between 6 K and 10 K the shape of the EPR spectrum of PS I does not change. The signal of X is characterized by a very fast spin-lattice relaxation rate. For example, at 7 K the signal begins to saturate at a microwave power of 100 mW.

(ii) At temperatures higher than 10 K, the shortening of T_1 leads to an increase of the width of the

X signal which results from a Lorentzian broadening of the spin-packet lines. The half-width $\delta_L(T)$ of these packets is then proportional to $1/T_1$. It was deduced from the experimental data by a procedure based on the methods presented in Refs. 14 and 26: the low-temperature (unbroadened) peak at $g = 1.78$ of half width δ_0 was numerically convoluted with a Lorentzian line of half-width δ_L, and the half-width δ of the resulting peak was plotted as a function of δ_L (Fig. 2a). The relaxation component $\delta_L(T)$ was then deduced from the comparison of the width $\delta(T)$ measured on the experimental spectrum with the calculated curve of Fig. 2a. The variations of $\delta_L(T)$ in the range 10 K to 21 K are represented in Fig. 2b. These variations are well fitted by the relation

$$\delta_L \propto e^{-\Delta/k_b T}$$

with $\Delta = 50$ cm^{-1}. This law corresponds to an Orbach relaxation process involving an excited state of energy Δ [14].

(iii) The temperature dependence of the intensity of the EPR signal of center X, as measured by the area of the $g = 1.78$ peak, was studied between 6 K and 21 K. No significant deviation from the Curie law could be detected in this temperature range. In what follows, we show that the remarkable properties of center X are well explained if X is a 2Fe-2S cluster characterized by weak antiferromagnetic exchange interactions.

In a reduced 2Fe-2S cluster, the Fe(III) ($S_1 = 5/2$) and Fe(II) ($S_2 = 2$) ions are coupled by an exchange interaction $-2JS_1 \cdot S_2$, and the energy of the first excited level is given by $\Delta = -3J$ (Fig. 3). Our measurements would give $J \approx -17$ cm^{-1} for center X. With this value, the expected deviation from the Curie law is 5% at 20 K, which could not be detected with the precision of our intensity measurements.

We have shown previously that when $|J|$ is small, the fine structure terms

$$D_i[S_{iz}^2 - \tfrac{1}{3}S_i(S_i+1)] + E_i[S_{ix}^2 - S_{iy}^2] \quad i = 1, 2$$

of the two iron atoms mix significantly the $S = 3/2$ excited states with the ground doublet (Fig. 3), so that the $\tilde{g}$ tensor of the doublet becomes a func-
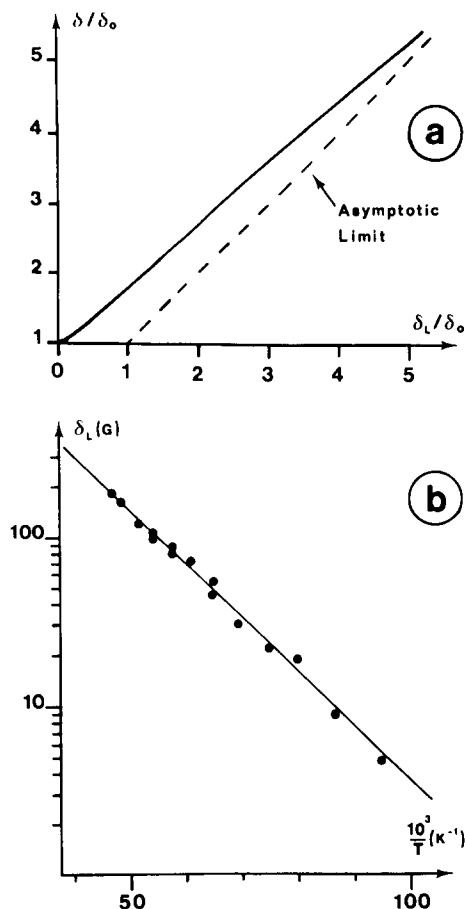
Fig. 2. (a) Variations of the half-width δ calculated by convolution of the low-temperature peak at $g = 1.78$ with a Lorentzian line of half-width δ_L. δ_0 is the residual half-width measured below 10 K. (b) Temperature dependence of the spin-lattice relaxation broadening component δ_L of center X measured on the $g = 1.78$ peak. The theoretical fit corresponds to $\delta_L \propto \exp(-\Delta/k_B T)$ with $\Delta = 50$ cm^{-1}.

tion of $\tilde{g}_1$, $\tilde{g}_2$, $\tilde{D}_1$, $\tilde{D}_2$ and J [15]. To estimate this effect in the case of center X, we use the following model:

(a) The tensors $\tilde{g}_1$ of Fe(III) and $\tilde{g}_2$ of Fe(II) are deduced from typical g-values of a 2Fe-2S plant ferredoxin:

$$g_x = 1.890, \quad g_y = 1.958, \quad g_z = 2057$$

by using the model presented in Ref. 16. In the 2Fe-2S cluster of plant ferredoxins, $|J|$ is large and the contribution of the fine structure terms can be neglected [15].
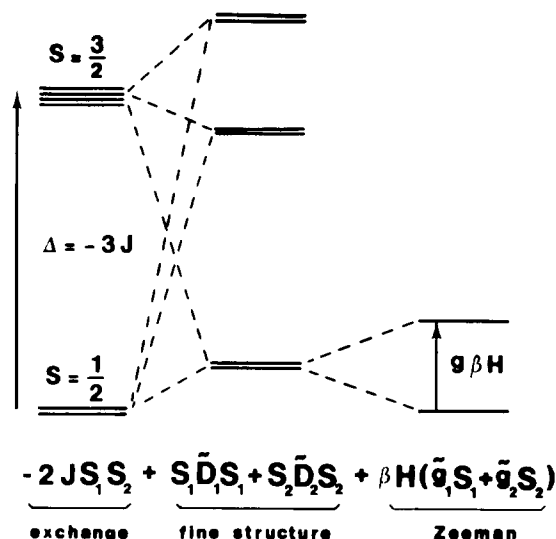

Fig. 3. Representation of the low-lying levels of a Fe(III)-Fe(II) high spin pair coupled by an exchange interaction.

(b) For the fine structure tensor $\tilde{D}_1$ of Fe(III), we use the values $D_1 = 1.4$ cm^{-1}, $E_1/D_1 = 0.25$ measured in oxidized rubredoxin, where a single Fe(III) atom is coordinated to four sulfur atoms [17].

(c) The value $-J = 17$ cm^{-1} is deduced from our relaxation broadening study.

A good agreement with the experimental g-values of center X ($g_x = 1.78$, $g_y = 1.88$, $g_z = 2.08$) can then be obtained for reasonable values of the fine structure components of the Fe(II) atom. For example, we obtain

$$g_x = 1.78, \quad g_y = 1.89, \quad g_z = 2.07$$

with $D_2 = 5.5$ cm^{-1}, $E_2 = 0$ (Fig. 4).

According to the model of Ref. 16, the x-axis corresponding to the lowest component of the $\tilde{g}$-tensor is perpendicular to the iron-iron axis of the binuclear cluster. An EPR investigation of magnetically oriented spinach chloroplasts has concluded that the axis corresponding to $g = 1.78$ is perpendicular to the membrane [18]. Our model then supports a structure of PS I where the iron–iron axis of center X is parallel to the membrane plane.

It is interesting to note that no acceptable fit of the g values could be obtained by using the $\tilde{g}_2$ tensor of the Rieske type 2Fe-2S clusters, where one iron atom is coordinated to noncysteine
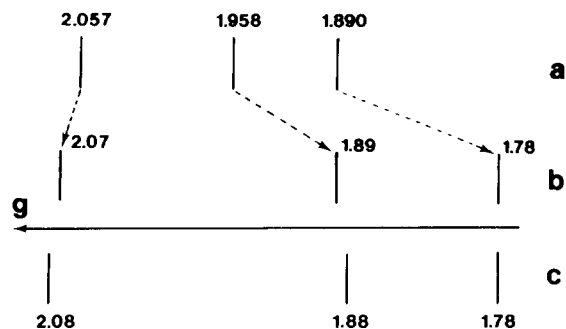
Fig. 4. Representation of the fine structure contribution to the g-tensor components of center X. (a) Experimental g-values of a typical [2Fe-2S] plant ferredoxin. (b) g-tensor of X center calculated as explained in the text with $-J = 17$ cm^{-1}. (c) Experimental g-values of center X.

ligands [19]. Thus our model does not support the proposition of a structure of this type for center X [10].

In metalloproteins, the low-temperature broadening of the EPR lines is attributed to a 'g strain' effect, which reflects a distribution of conformations of the paramagnetic cluster [20]. We have shown previously that in iron-sulfur clusters characterized by low $|J|$ values, the distribution of the exchange and fine structure parameters also contribute to the line-width, so that the 'g strain' effect is much increased [21,22]. This could explain the anomalous linewidths observed in the spectrum of center X (fig. 1).

Our model accounts for the remarkable EPR properties of center X in the reduced state: a very fast spin-lattice relaxation rate, a highly anisotropic $\tilde{g}$-tensor and very broad EPR lines. All these properties are the consequence of the low value $|J| \approx 17$ cm^{-1}, which is much smaller than the values $|J| \geq 90$ cm^{-1} usually measured in reduced 2Fe-2S clusters [23-27]. By analogy with what is observed in oxidized 2Fe-2S model compounds [28,29], this important lowering of $|J|$ could indicate that the bridging sulfide are involved in additional bonds, so that the iron-iron distance could be increased by comparison with the usual 2Fe-2S clusters.

The small $|J|$ value of the reduced state leads us to suggest an attractive explanation for the very negative redox potential of center X. If the value of the exchange parameter in the oxidized state were similar to that measured in plant ferredoxins,

$J \approx -180$ cm^{-1} [23,24], the large variation of J between the two redox states would lead to an important stabilization of the oxidized form and a corresponding lowering of the redox potential compared to the value of about -420 mV measured in plant ferredoxins. By using the model presented in Ref. 30, it is possible to show that this lowering could be as large as 200 mV.

We have shown that the theoretical models which describe the magnetic properties of the reduced 2Fe-2S clusters can be used to account quantitatively for the remarkable properties of center X. At the present time, no equivalent theoretical model can be applied to the reduced 4Fe-4S clusters, so that the hypothesis of a 4Fe-4S structure cannot be a priori rejected. Nevertheless, we believe that the good overall consistency of our interpretation supports the proposal of a 2Fe-2S structure for center X of PS I.

Our study gives no indication concerning the number of clusters corresponding to center X, and detailed EPR intensity measurements are in progress to elucidate this point.

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