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ON THE INTERACTION AND ORIENTATION OF THE IRON-SULFUR CENTERS A AND B IN CHLOROPLASTS OF HIGHER PLANTS

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The iron-sulfur centers A and B of spinach and barley chloroplasts were studied using EPR spectroscopy. The spectrum of samples with both centers reduced is significantly different at the microwave frequencies 9 and 35 GHz. This shows that an interaction exists between the centers which is discussed in terms of exchange and dipolar effects. The orientation of the *g* tensors of centers A and B was studied in magnetically oriented chloroplasts. Changes were observed in going from the partially to the fully reduced sample, a fact which strengthens the interaction model. The existence of an interaction implies that the centers are situated close to each other, presumably in the same molecule and in the same electron-transport chain.

Introduction

Iron-sulfur proteins play an important role in the electron transport of photosynthetic systems in higher plants. On the acceptor side of Photosystem I there are three Fe-S centers, X, the nature of which is largely unknown, and the much studied centers A and B [1]. In addition, the so-called Rieske Fe-S center functions in the electron transport between the two photosystems. The optical absorption of these centers is relatively weak, but they can be readily studied in their reduced form by EPR [1].

In a dark-adapted sample, frozen in the dark and illuminated at low temperature, one electron is moved from *P*-700 in an irreversible reaction leading to the reduction of one acceptor per reaction center. In this way, center A is predominantly reduced, although reduction of center B is also clearly observed in barley and certain blue-green algae [2]. On the other hand, both centers A and B can be reduced if a sample is illuminated before and during freezing, particularly if a strong reductant such as dithionite is present. The EPR spectrum of center A is not the same in both cases and it has been suggested that interaction between the two centers occurs [1,2].

The orientation of the Fe-S centers relative to the photosynthetic membrane can be studied by EPR in samples containing ordered membranes. Such studies have been performed by Dismukes and Sauer [3] on magnetically ordered chloroplasts and by Prince et al. [4] on chloroplasts oriented on mylar sheets. Although interaction between centers A and B was discussed in Ref. 4, the orientation dependence was in both cases interpreted using a model in which centers A and B are independent of each other.

In this paper, the possible magnetic interaction between the Fe-S centers A and B has been studied by EPR investigations at a higher frequency, a possibility also pointed out by Prince et al. [4] and previously used for a bacterial ferredoxin [5]. The orientation of the Fe-S centers in magnetically oriented membranes has also been reexamined, and the orientation of centers A and B in a partially reduced sample has been compared to the orientation in the fully reduced case. Also, some implications for the role of centers A and B are discussed.

Abbreviation: Tricine, *N*-tris(hydroxymethyl)methylglycine.

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Materials and Methods

Broken barley or spinach chloroplasts were prepared in the following way. Leaves were ground in a Waring Commercial Blendor for two periods of 5 s in a grinding medium consisting of 350 mM sucrose, 50 mM Tricine-KOH, pH 7.5, 10 mM NaCl and 0.5 mM EDTA. After centrifugation, the pellet was resuspended in a medium consisting of 50 mM Tricine-KOH, pH 7.5, 10 mM NaCl and 0.2 mM EDTA. The solution was again centrifuged and the chloroplast fragments were resuspended in the same medium to a final chlorophyll concentration of approx. 7 mg/ml. For orientation studies ethylene glycol was added (25%, v/v). The chloroplast membranes were oriented by exposing the samples for 2 min to a magnetic flux density of 1.4 T before freezing in the magnetic field at liquid nitrogen temperature.

Reduction of both centers A and B was accomplished by the addition of 5 mM sodium dithionite before freezing under exposure to intense white light. Reduction of essentially only center A was obtained by freezing dark-adapted samples followed by illumination at low temperature, in most cases directly in the EPR cavity.

EPR X-band spectra were recorded with a Varian E-9 spectrometer equipped with an Oxford Instruments ESR-9 helium flow cryostat. 35 GHz spectra were run at about 20 K with a Varian V-4503 spectrometer using a home-built cryostat [6]. The E-9 spectrometer was connected on-line to a Nova 3 minicomputer, by which data treatment such as signal averaging and obtaining of difference spectra could be performed. The computer program was written in Basic. The 35 GHz spectra were fed via a curve reader (a modified HP 7004 *X-Y* recorder) to the same minicomputer.

Results

Fig. 1 shows the 9 and 35 GHz spectra of a dithionite-reduced sample illuminated during freezing. Presumably, both centers A and B are reduced. The horizontal scale is chosen so that the spectra have a common *g*-value scale. Thus, if both centers have $S = 1/2$ and no interaction, all peaks should appear at the same position in the two spectra. Clear differences are observed, however, particularly in the region *g* 2.04–2.07. The 35 GHz spectrum also shows a distinct shoulder at *g* 1.86, and the *g* 1.92 derivative-type line is shifted. On the other hand, a sample having essentially only center A reduced showed peaks at exactly the same *g* value at both frequencies (not shown).

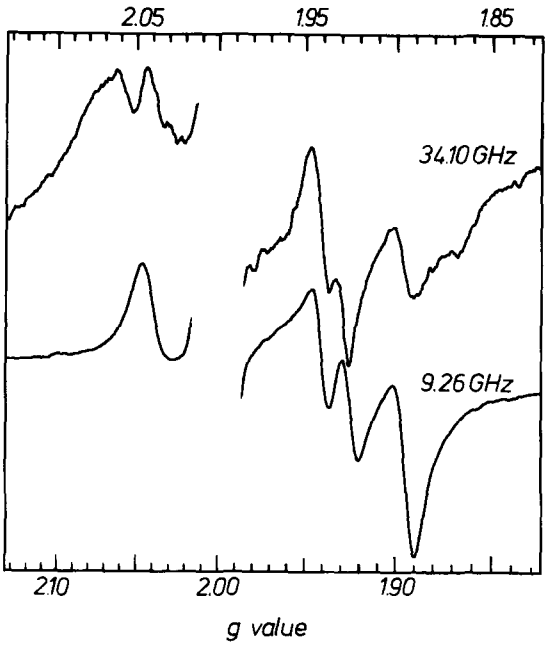

Fig. 1. EPR spectra at two microwave frequencies of barley chloroplasts reduced with dithionite and frozen under illumination. Microwave frequency and power: (A) 34.10 GHz and 2 mW; (B) 9.260 GHz and 6 mW; respectively. The spectra were reproduced on the same *g*-value scale. Temperature, 18 K.

Since a magnetic interaction might produce an unusual temperature dependence of the EPR spectra, samples were studied in the temperature range 12.5–35 K (Figs. 2 and 3). The spectrum from a sample illuminated at low temperature only (Fig. 2) shows the presence of three Fe-S centers, center A (*g* 1.87, 1.95, 2.05), the Rieske center (with a peak at *g* 1.9) and a minority component (*g* 1.88, 1.93, 2.07) to be discussed below (cf. Fig. 4). The center A signal broadens more at higher temperature than the other signals, which causes the spectrum to change its general appearance, but no anomalous temperature dependence can be observed. The same is true for the fully reduced sample (Fig. 3).

EPR spectra of oriented samples with different

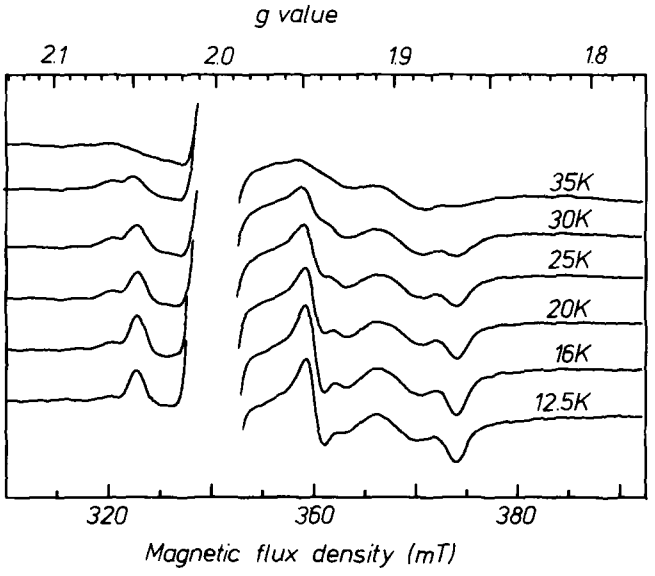

Fig. 2. Temperature dependence of EPR spectra from dark-adapted spinach chloroplasts illuminated at low temperature. The spectra were recorded at nonsaturating microwave power and normalized with respect to power (P) and temperature (T) through multiplication by $T/\sqrt{P}$. Microwave frequency, 9.25 GHz.

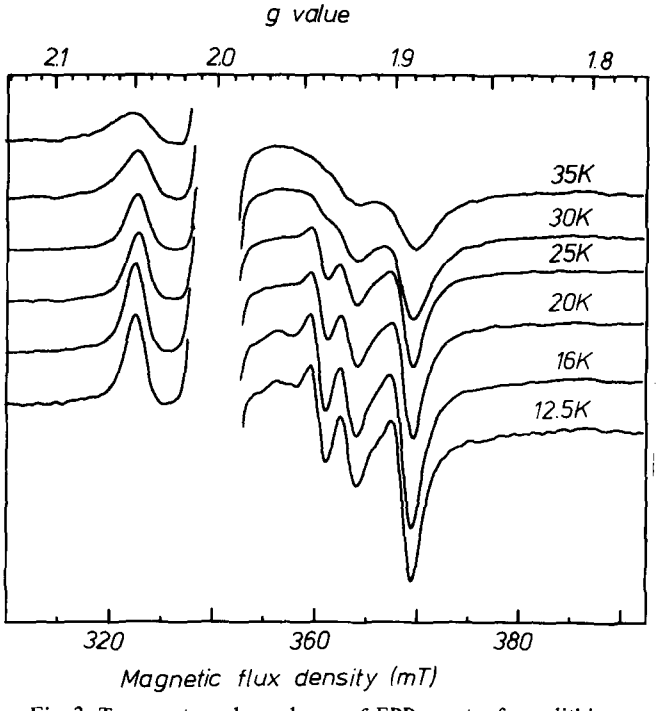

Fig. 3. Temperature dependence of EPR spectra from dithionite-reduced spinach chloroplasts illuminated during freezing. The spectra were recorded and normalized as in Fig. 2.

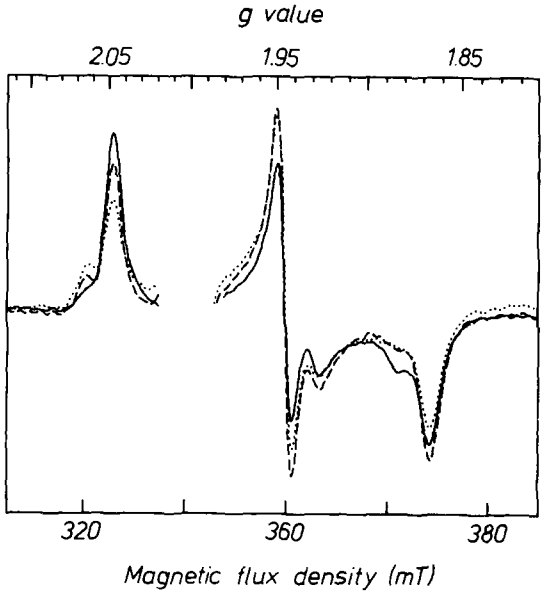

Fig. 4. EPR spectra of oriented spinach chloroplasts. The differences between signals from dark-adapted samples before and after illumination at 18 K are presented, which eliminates the contribution from the Rieske center. The angles given are those between the spectrometer magnetic field and the membrane normal. The microwave frequency and power were 9.25 GHz and 6 mW. (· · · · · ·) 0°, (- - - - - -) 45°, (———) 90°.

TABLE I

g VALUES AND ORIENTATIONS OF Fe-S CENTERS

The angles ϕ are those at which a particular peak has its maximal amplitude.

		x	y	z
Partially reduced				
Center A	g	1.87	1.95	2.05
	ϕ	30–45	30–45	75–90
Center B	g	1.88	1.93	2.07
	ϕ	80–90	80–90	0–15
Fully reduced				
Centers A + B	g	1.89	1.92 1.94	2.05
	ϕ	90	0 40–60	30–50
Center A [a]	g	1.89	1.94	2.05
	ϕ	90	40–60	≈0
Center B [a]	g	1.89	1.92	2.04
	ϕ	90	0	≈90

[a] The assignment of centers A and B of the fully reduced sample may be hazardous, as discussed in the text.

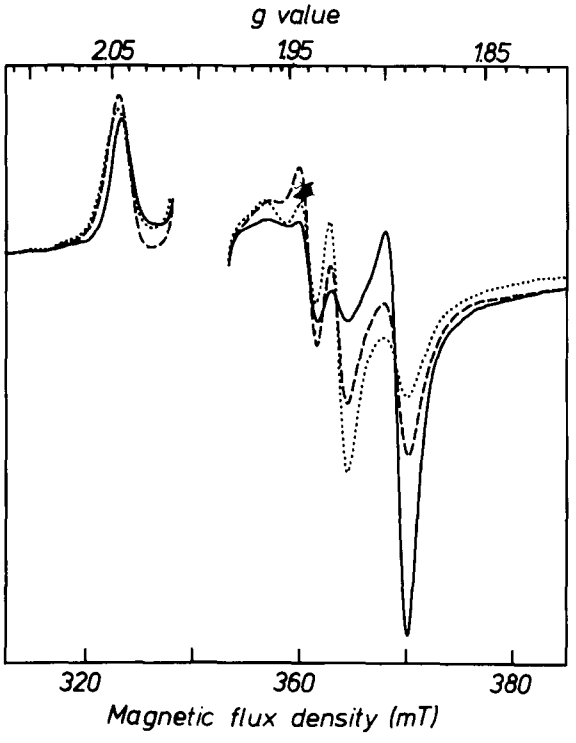

Fig. 5. EPR spectra of oriented spinach chloroplasts reduced with dithionite and illuminated during freezing. Conditions were as in Fig. 4.

degrees of reduction of the Fe-S centers are shown in Figs. 4 and 5. The nomenclature used is that of Dismukes and Sauer [3], i.e., 0° means that the spectrometer field is parallel to the membrane normal. Table I shows the values of the angles at maximum signal amplitude obtained from an analysis at intermediate angles.

The behavior of spinach and barley chloroplasts is the same apart from a line of unknown origin appearing at g 1.96 in the spectra of fully reduced chloroplasts from spinach. Furthermore, the degree of orientation obtained is significantly smaller with barley than with spinach chloroplasts.

Discussion

Interaction between Fe-S centers

Although the differences between the two spectra recorded at 9 and 35 GHz of the fully reduced sample (Fig. 1) are small, they are quite significant. It should be pointed out that the spectra are recorded with the same sample tube, which eliminates the possibility that the differences are due to incomplete reduction of the 35 GHz sample. Thus, the centers do not behave as isolated $S = 1/2$ systems. A close examination of the spectra at 9 and 24 GHz published by Hoff [7] does in fact reveal the same frequency dependence, although he concluded that no interaction exists.

The effect of increasing the microwave frequency is certainly much less in the present case than for the bacterial ferredoxins studied by Mathews et al. [5]. In fact, at 35 GHz the spectrum has an appearance more like that expected with centers A and B reduced in separate reaction centers with no interaction (cf., for example, Ref. 8 and Fig. 4). An exchange coupling with a magnitude about equal to the frequency difference at 9 GHz between the two centers (i.e., 0.01 cm^{-1}) might be responsible for both the difference between the 9 and the 35 GHz spectra in Fig. 1 and for the shifts in peak position at 9 GHz between the partially and the fully reduced state. The exact position of the peaks at 9 GHz would be dependent on the relative orientation of the g tensors. Of course, the presence of a dipolar coupling is not excluded, but it is not likely to be much greater than 10 mT, corresponding to a minimum distance of 5–6 Å between the centers. Another possibility is that part of the shifts observed in going from the partially to the fully reduced state is caused by a conformational interaction. The reduction of one center would then lead to an altered ligand arrangement around the other. However, this mechanism would give two isolated $S = 1/2$ centers and thus cannot explain the difference between the two spectra in Fig. 1. It is also unlikely as judged from experiments with chloroplasts from algae [2] in which irradiation of a frozen sample induces the shifts.

An interacting system shows anomalous temperature dependence in the region where kT is equal to the interaction energy (see, for example, Ref. 5). However, Fig. 2 shows no anomaly in the behavior of the sample with both centers A and B reduced when $9 < kT < 24$ cm^{-1}. This is particularly true if a comparison is made with Fig. 3, which shows a temperature study of a sample irradiated at low temperature. Here, only one electron per reaction center leaves P-700 so that only one Fe-S center can be reduced

which leads to a noninteracting system. In fact, this sample shows a greater change in shape due to the presence of a minority center (to be discussed below) which broadens less at higher temperature. Thus, the temperature dependence is entirely consistent with a very small interaction energy.

Orientation dependence of Fe-S centers

The degree of orientation of the membrane fragments in this work appears to be considerably higher than that obtained in Ref. 3 in which the same magnetic field technique was used. The reason for this is unclear, since it can only in part be due to the higher field strength used here. On the other hand, dehydration on mylar sheets as in Ref. 4 gives a higher degree of order. It is of interest to compare the result of the latter technique with our data, which involve a less drastic treatment of the sample with better control of its chemical state.

Dark adaptation followed by illumination at low temperature yields an EPR spectrum mainly arising from center A (Fig. 4). The orientation is essentially the same as that found by Prince et al. [4], with the difference in orientation of the axes being of the order of 10–20°. In addition to the signal from center A, the illumination induces three other peaks in the spectrum (g 1.88, 1.93, 2.07) (Fig. 4). From their magnitude and orientation dependence (see Table I), they appear to arise from a single Fe-S center. We associate this signal with center B as has also been done by other groups [2,8]. This means that there is a small probability that the electron leaving *P*-700 will enter center B rather than center A. In the work by Prince et al. [4] this spectrum may be masked by the signal from some reaction centers in which both centers A and B are reduced. This could also explain why they detect a peak at g 2.04, which we detect only at full reduction (see below).

For the sample with both centers A and B reduced (Fig. 5) we again have data in general agreement with those of Prince et al. [4]. However, we also have information on the orientation dependence of g 2.05, since it is not masked by a plastocyanin copper signal. This peak shifts with orientation, probably because it consists of two components, one at g 2.05 with maximal intensity at an angle not far from 0° and another at g 2.04 with a maximum closer to 90°. The interaction demonstrated above may make it meaningless to associate individual peaks in this spectrum with particular Fe-S centers. In addition, the orientation dependence may no longer be that of $S = 1/2$ systems, although the spectrum can be simulated on the basis of noninteracting centers [9]. An assignment along the traditional lines [1] has been tried, however, and is shown in Table I. The assignment of the g 2.04 and 2.05 peaks is made so that the sum of $\cos^2\phi_i$ for $i = x, y$ and z is reasonably close to unity (cf. Ref. 4) for both centers. Note that the orientation of both centers A and B is different in the partially and fully reduced samples.

General significance of the interaction

Exchange and/or dipolar interaction between the two Fe-S centers A and B does exist, since they do not behave as isolated $S = 1/2$ spin systems. An assignment based on independent centers would imply changes not only in g values as observed earlier [1], but also in the orientation of the g tensors in going from the partially to the fully reduced state. This strengthens the notion of interacting centers. They must be fairly close for this interaction to occur, and most likely they reside in the same molecule in the same way as the two centers in the 8-iron ferredoxins [5]. This implies that centers A and B are members of the same electron-transport chain, presumably taking part in a sequential electron transfer. It has previously been suggested that one of the centers takes part in noncyclic and the other in cyclic electron flow [10], but this possibility appears to be very unlikely in the light of the present results.

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