Biochimica et Biophysica Acta, 592 (1980) 323—337

BBA 47895

THE ORIENTATION OF THE MAGNETIC AXES OF THE MEMBRANE-BOUND IRON-SULFUR CLUSTERS OF SPINACH CHLOROPLASTS

ROGER C. PRINCE *, MARK S. CROWDER and ALAN J. BEARDEN

Department of Biophysics and Medical Physics and Division of Biology and Medicine, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720 (U.S.A.)

(Received January 14th, 1980)

Key words: EPR; Rieske center; Iron-sulfur cluster orientation; Photosystem I; (Chloroplast membrane)

Summary

Spinach chloroplast membranes were oriented onto mylar sheets by partial dehydration, and the orientation of the magnetic axes of membrane-bound paramagnetic clusters determined by electron paramagnetic resonance (EPR) spectroscopy. Our results indicate that the reduced Rieske iron-sulfur cluster signal is of orthorhombic symmetry oriented with the $g_y = 1.90$ axis orthogonal to the membrane plane and with the $g_z = 2.03$ axis in the membrane plane; the g_x-axis is undetectable, presumably due to its broadness. If the Rieske center is a two-iron iron-sulfur cluster, we conclude that the iron-iron axis lies in the plane of the membrane.

Illumination reduces the two bound chloroplast iron-sulfur proteins known as Clusters A and B. Center A is oriented such that $g_x = 1.86$ and $g_y = 1.94$ lie at an angle of about 40°, and $g_z = 2.05$ is at approximately 25°, to the membrane plane. There are two possible orientations of Cluster B depending on the set of g-values assigned to this cluster. For one set of g-values, $g_z = 2.04$ and $g_x = 1.89$ are oriented in the plane of the membrane while $g_y = 1.92$ is orthogonal to the plane. Alternatively, $g_z = 2.07$ and $g_y = 1.94$ are oriented approximately 50° and 40° to the membrane plane respectively, and $g_x = 1.80$ is in the plane of the membrane. An additional light-induced signal at $g = 2.15$ oriented orthogonal to the plane is currently unexplained, as are other membrane perpendicular signals seen at $g = 2.3$ and $g = 1.73$ in dark-adapted samples.

Introduction

Membrane-bound iron-sulfur clusters are found in many energy conserving systems, including bacteria, mitochondria, and chloroplasts. Plant and animal

* Permanent address to which correspondence should be addressed: Department of Biochemistry and Biophysics/G3, University of Pennsylvania, Philadelphia, PA 19104, U.S.A.

Abbreviation: DBMIB, 2,5-dibromo-3-methyl-6-isopropylbenzoquinone.

mitochondria, together with many bacteria, contain a plethora of distinct iron-sulfur clusters which have been resolved by their thermodynamic and spectral characteristics (see Ref. 1 for a recent review). In contrast, chloroplast membranes have been reported to contain only three, or perhaps four distinct iron-sulfur species (see Ref. 2). One of these [3] is similar in its properties to the so-called 'Rieske cluster' of mitochondria and bacteria [4,5]. In chloroplasts this cluster is characterized by a prominent EPR signal at $g = 2.03$ and 1.90; it apparently functions between plastoquinone and cytochrome *f* in the electron flow sequence between the two photosystems [6]. Two other iron-sulfur clusters are associated with the reducing end of Photosystem I, and may function to deliver electrons to NADP. Both have rhombic EPR characteristics and the two are known as iron-sulfur Clusters A and B. Cluster A is irreversibly reduced, with the concomitant generation of oxidized Photosystem I reaction center chlorophyll, when dark-adapted chloroplasts are illuminated at cryogenic temperatures [7]. It is distinguished by an EPR spectrum with $g_x = 1.86$, $g_y = 1.94$ and $g_z = 2.05$. Cluster B is also photoreducible at low temperature [8], but this occurs to an appreciable extent only if Cluster A is prereduced. Reduction of Cluster B can be achieved either by chemical reduction [9,10] or by intense illumination during the freezing process [8]. One noteworthy feature of the lineshapes of the EPR spectra of the two iron-sulfur proteins is that when Cluster B is reduced, a spectrum characterized by *g*-values at 2.05, 1.94, 1.92 and 1.89 is observed, while the signal at $g = 1.86$ attributed to Cluster A essentially disappears. Although this effect is not fully understood, it has been suggested that the $g = 1.86$ band reappears as part of the $g = 1.89$ band as a result of spin-spin interaction between the two paramagnetic reduced iron-sulfur clusters. The equilibrium oxidation-reduction potentials of iron-sulfur Clusters A and B are sufficiently negative that they cannot be stably chemically reduced in aqueous buffers at neutral pH, but they can be titrated at alkaline pH (e.g., pH 10), where E_m values of about −550 mV have been obtained [9].

Another EPR detectable component has been observed to function in Photosystem I primary reactions. This component known as X [12] is characterized by *g*-values of $g_z = 2.07$, $g_y = 1.86$ and $g_x = 1.76$ [13,14]. Intense illumination during freezing, even in the absence of strong reductants and high pH, captures this component in its EPR observable reduced state. This component is also photoreducible at cryogenic temperatures but Clusters A and B must be chemically prereduced; under these conditions the photoreduction of X is a reversible process.

In the work presented here we examine the orientation of the magnetic axes of the Rieske cluster and the two bound iron-sulfur proteins A and B in chloroplast membranes which have been aligned by partial dehydration on a mylar sheet. We find that all three show a high degree of orientation within the membrane.

Materials and Methods

Whole spinach chloroplasts were prepared by the method of Malkin [15] using the following blending solution; 300 mM sucrose, 50 mM Tris-HCl

(pH 7.8), 10 mM NaCl, and 1 mM Na_2-EDTA (pH 7.8). Washed, broken chloroplasts were prepared by resuspending the whole chloroplasts in a 1 : 10 dilution of the blending solution. Free manganese was removed by two washings in the hypotonic solution. Broken chloroplast membranes were oriented by layering a concentrated suspension onto collodion coated mylar, and then partially dehydrating the sample in a 90% relative humidity atmosphere for 24—36 h at 4°C [16]. Alternatively, the partial dehydration was sometimes attained by evaporation under a stream of nitrogen gas [17].

X-band EPR spectra were obtained at cryogenic temperatures with a modified JEOL ME-IX spectrometer with a TE_{011} cavity. First-derivative spectra were recorded using 100 KHz magnetic-field modulation and a linear phase-sensitive detector system operating at 100 KHz. Field modulation amplitudes

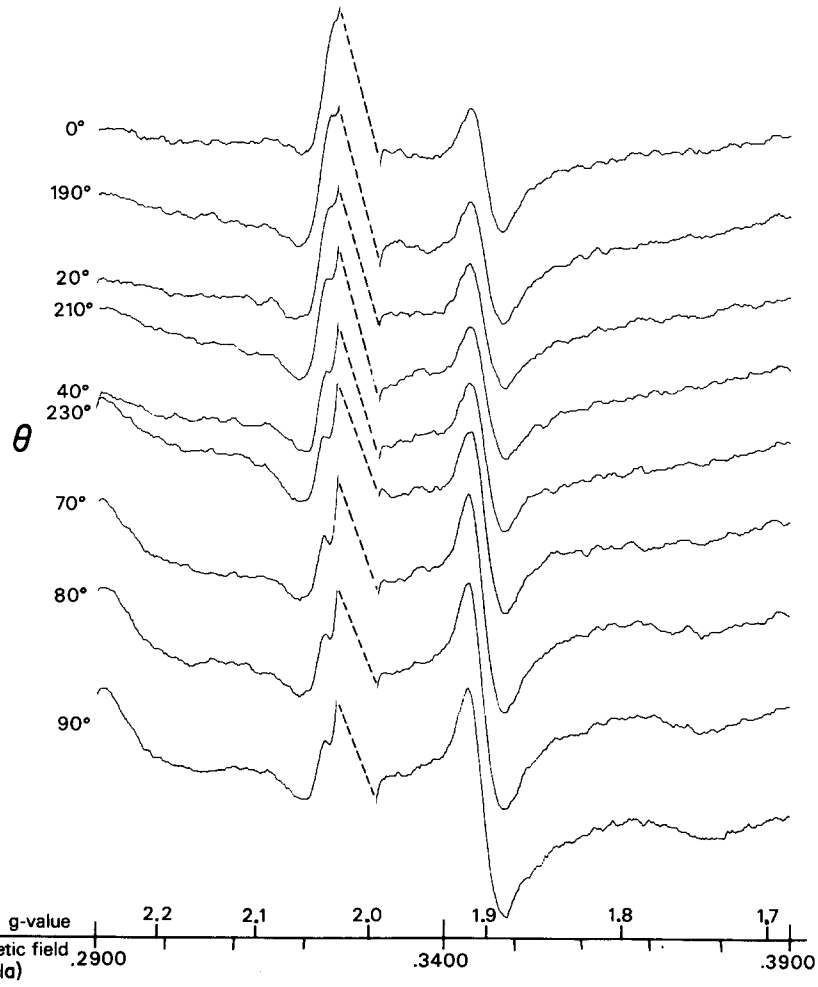

Fig. 1. EPR spectra recorded in the dark at 16 K of oriented multilayers of broken spinach chloroplasts. The angles refer to the direction of the magnetic field (H_0) relative to the membrane plane, such that at 0°, H_0 and the membrane plane are parallel. EPR-operating conditions: microwave power, 15 mW; modulation amplitude, $1.25 \cdot 10^{-3}$ Tesla.

were in the range of $0.16 \cdot 10^{-3}$—$1.25 \cdot 10^{-3}$ Tesla. To enhance sensitivity, the conventional klystron microwave power source was replaced by a low noise Gunn-diode oscillator (Central Microwave). A double-balanced mixer (RHG, DM8-128), functioning as a homodyne phase detector, further complemented the spectrometer.

The DBMIB was a gift from Dr. Richard Malkin.

Results

Fig. 1 shows the EPR spectra of oriented chloroplast membranes in the dark, with the angle (θ) between the applied magnetic field and the membrane planes indicated at the left of the figure. It is important to note that since the membranes dry onto the mylar, the plane of the membranes is parallel to the mylar support, and the normal to the membrane plane is normal to the mylar [16]. Thus, in the figures presented here, spectra taken at 0° and 180° were obtained with the membranes lying parallel to the spectrometer magnetic field. The dark-adapted oriented chloroplast membranes exhibited five EPR signals near the $g = 2$ region and all of these signals showed a marked angular dependence. The signals at $g = 1.90$ and $g = 2.03$ may be ascribed to the Rieske cluster; these axes are oriented such that the $g = 1.90$ axis lies perpendicular to the membrane plane, while the $g = 2.03$ axis lies parallel to the membrane (Fig. 2). A signal at $g = 2.3$ and another at $g = 1.73$, both of currently unknown origin, also have maximal intensity when the applied magnetic field is perpendicular to the oriented members. When DBMIB was added to the chloroplasts, the dark spectra showed a new signal at $g = 1.95$ (Fig. 3). It is orientation is parallel to the membrane plane (Fig. 4).

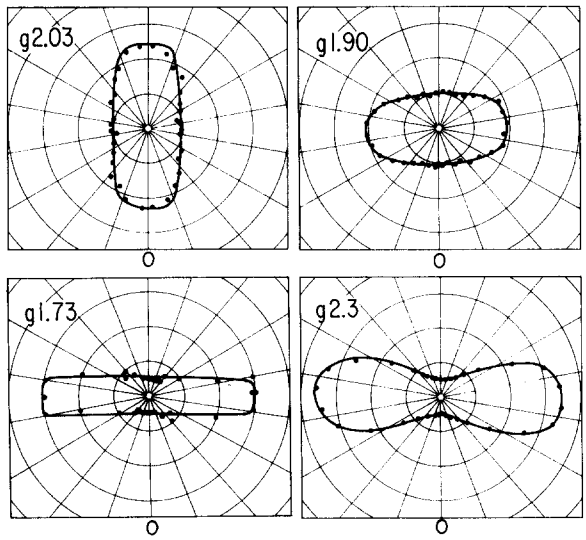

Fig. 2. Polar plots of the amplitudes of the signals shown in Fig. 1. Radial scale is in arbitrary units. 0° represents the angle when H_0 and membrane plane are parallel. Double plotting of points is obtained by transposing points in the 0°—180° region to the 180°—360° region and vice versa.

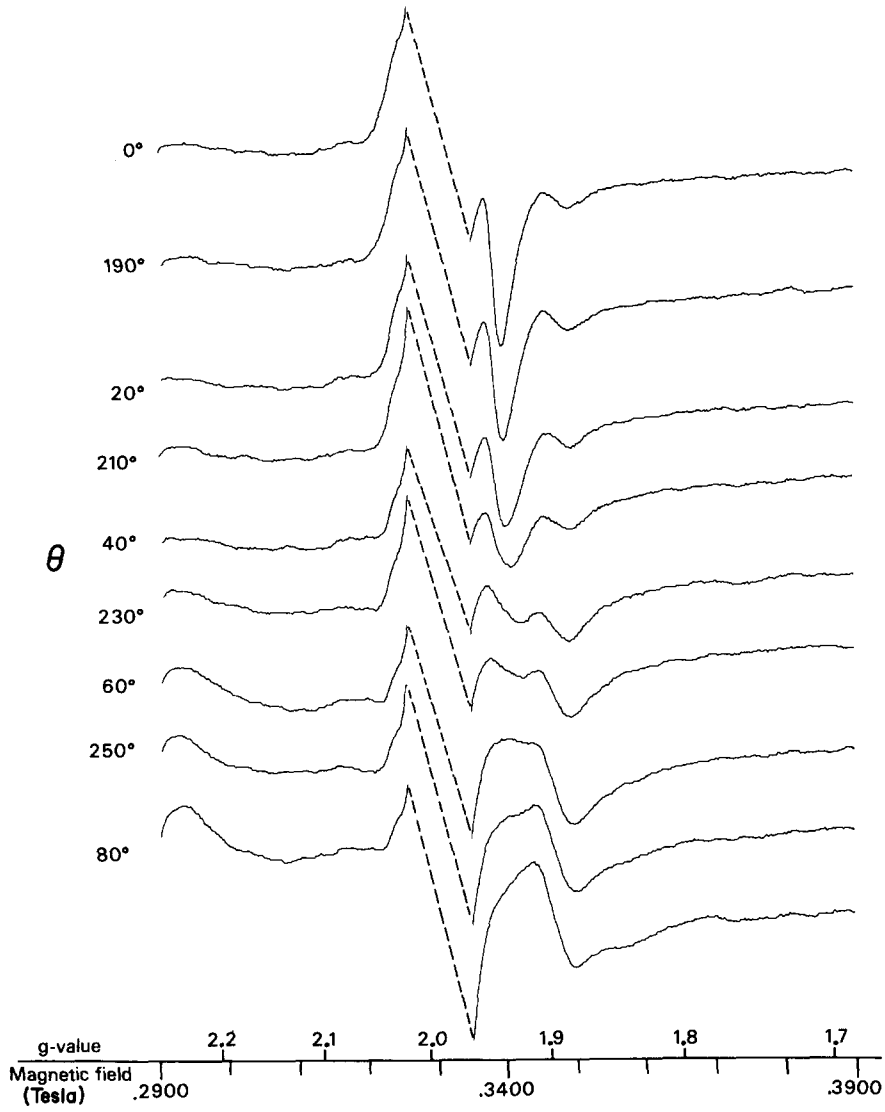

Fig. 3. Effect of DBMIB on the iron-sulfur Rieske center. Sample conditions are the same as in Fig. 1 except 30 μM DBMIB has been added. EPR-containing conditions are the same as in Fig. 1.

Fig. 5. shows the anguiar dependence of the light-induced EPR signals. In this case spectra were recorded at 10° intervals in the dark, the chloroplasts were illuminated with red light for several minutes in the spectrometer cavity, and another series of spectra were recorded at the same angles. The spectra shown in Fig. 5 represent 'light-minus-dark' difference spectra at the various angles, and show contributions from iron-sulfur Clusters A and B, and a previously unreported signal at $g = 2.15$. It is noteworthy that the spectral region near $g = 2.05$ can be resolved into three components of different angular dependence. The amplitudes of the major signals as a function of the angle between the applied field and the membrane plane are shown in Fig. 6.

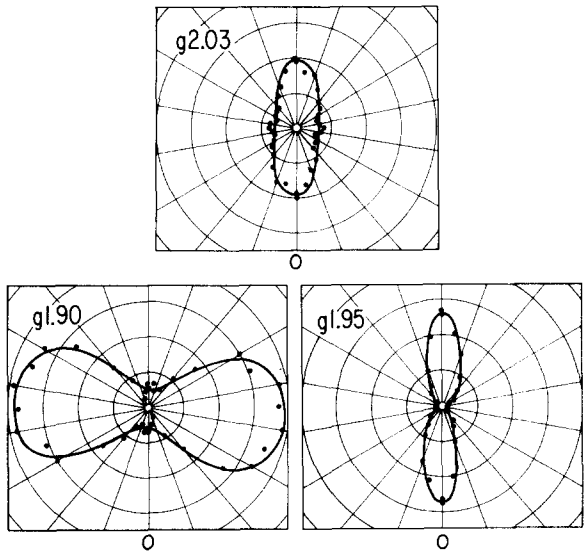

Fig. 4. Polar plots of the effects of DBMIB shown in Fig. 3. Radial scale in arbitrary units. Double plotting of points as done in Fig. 2.

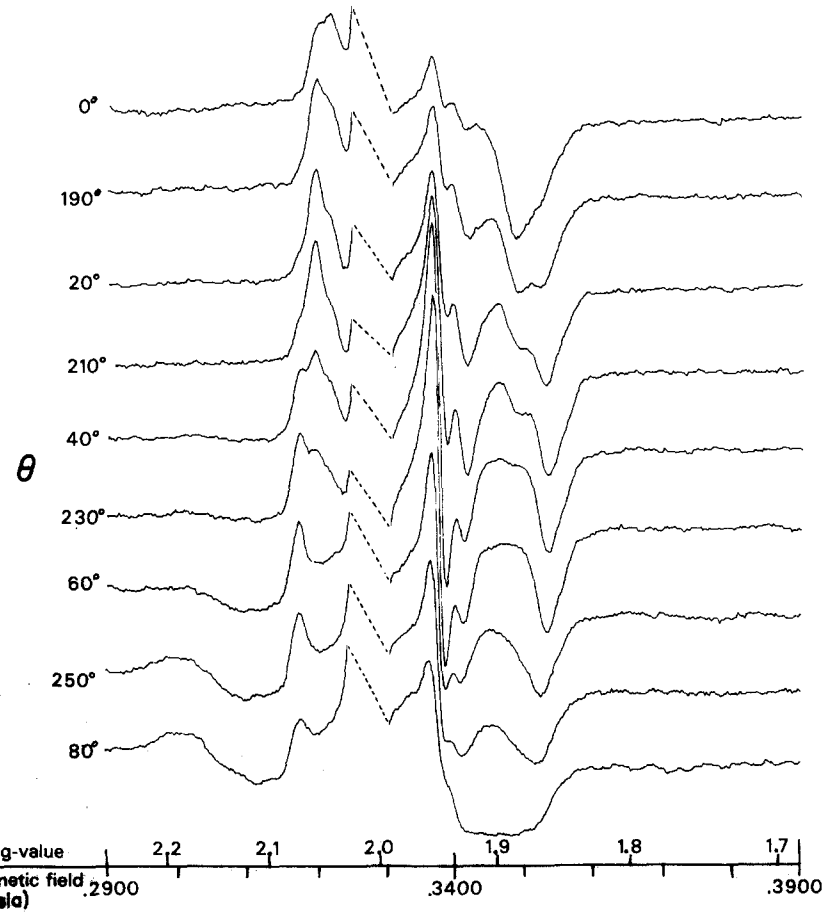

Fig. 5. Light-dark EPR spectra of oriented multilayers of broken spinach chloroplasts. Spectra were recorded at 10° intervals in the dark, the chloroplasts were illuminated with red light in the spectrometer cavity, and another series of spectra were recorded at the same angles. Light-dark spectra were obtained by subtraction of spectra at each angle shown. EPR-operating conditions: 16 K; microwave power, 15 mW; modulation amplitude, $1.25 \cdot 10^{-3}$ Tesla.

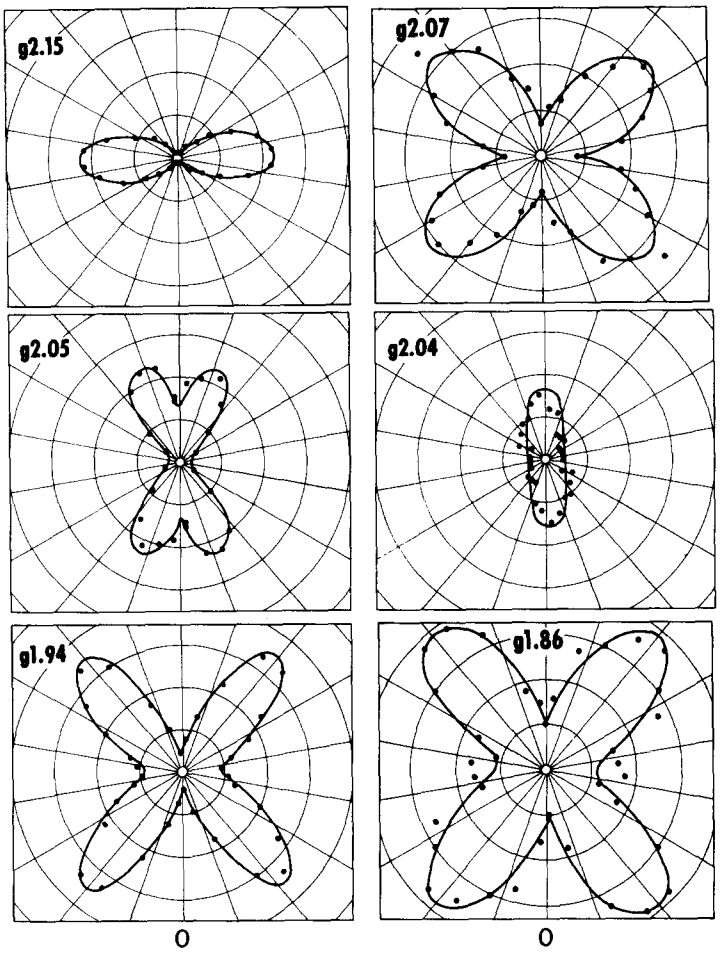

Fig. 6. Polar plots of the amplitudes of the signals shown in Fig. 5. Radial scale is in arbitrary units. Double plotting of points as in Fig. 2.

Illumination of oriented samples at cryogenic temperatures always partially reduced Cluster B with Cluster A (Fig. 5) even though unoriented samples taken from the same chloroplasts yielded only minute amounts of reduced Cluster B under identical conditions. Nevertheless, the small extent of Cluster B reduction in the oriented samples makes it difficult to resolve the angular dependence under these conditions. In order to determine the orientation of Cluster B, the experiment of Fig. 5 was repeated except that before the illumination the membranes were warmed to room temperature and illuminated with white light prior to and during refreezing. The results are shown in Fig. 7, which again shows 'light-minus-dark' difference spectra at each angle. While these membranes are apparently not as well oriented as those in Fig. 5 (for example, compare the 'dichroic ratio' of the $g = 1.94$ band), the orientation is qualitatively similar as shown by the angular dependence of the $g = 1.94$ band (cf. Figs. 6 and 8). The illumination before and during freezing clearly allows

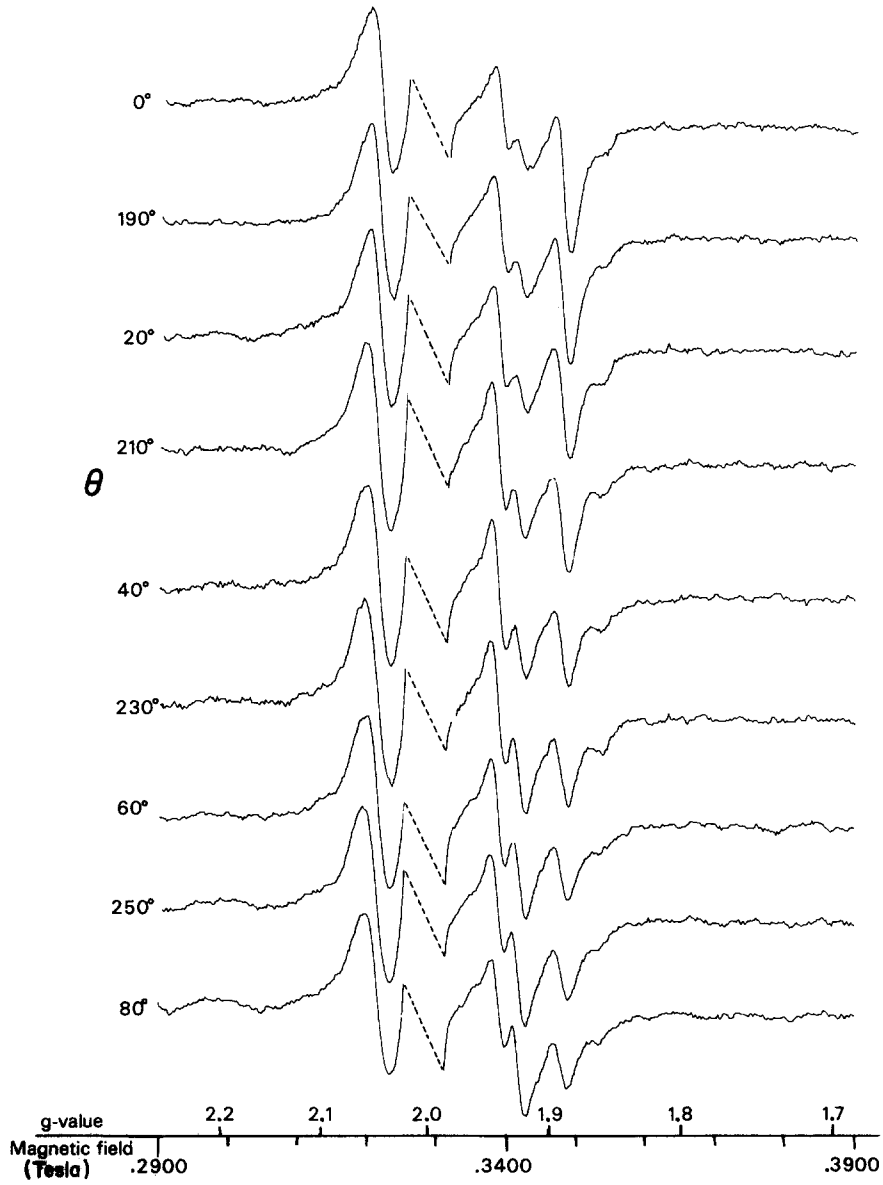

Fig. 7. Light-dark EPR spectra of oriented multilayers of broken spinach chloroplasts. Spectra were recorded at 10° intervals in the dark, chloroplasts were removed from the EPR cavity and warmed to room temperature then illuminated with white light during freezing. Another series of spectra were recorded at the same angles. Light-dark spectra was obtained by subtraction of spectra at each angle shown. EPR-operating conditions: 17 K; microwave power, 15 mW; modulation amplitude, $1.25 \cdot 10^{-3}$ Tesla.

more than one turnover of the Photosystem I reaction center, and there is now a substantial reduction of Cluster B with the Cluster A. The angular dependences of the $g = 1.92$ and $g = 1.80$ axes of Cluster B are shown in Fig. 8.

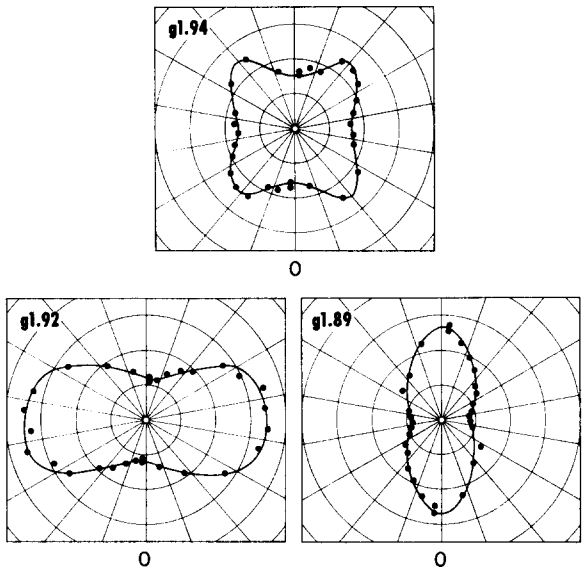

Fig. 8. Polar plots of the $g = 1.94$, $g = 1.92$, and $g = 1.89$ signals shown in Fig. 7. Radial scale is in arbitrary units. Double plotting of points as in Fig. 2.

Discussion

Oriented membranes vesicles have been widely studied in recent years (16—24). Such specimens approximate one dimensional crystals, being usually oriented relative to the membrane normal, but with no preferential orientation in the plane of the membrane. Any chromophores that have an orientation within the membrane therefore have orientation-dependent spectra in such systems [16,17,22—24]. Blum et al. [16,22] have shown that although the principal g-values of a crystalline paramagnetic center vary as the angle of the applied magnetic field is altered, even a small amount of disorder (called moisaic spread by Blum et al. [16,22]), about the predominant orientation leaves the g-values effectively constant. However, under these conditions the individual g-values show sharper derivative spectra, and the amplitudes of the individual g-values show an orientation dependence: the EPR spectra of most oriented biological membranes exhibit the latter behavior. In such systems the magnitude of the derivative EPR absorption of each g-value is maximal when the applied magnetic field lies parallel with the relevant axis of the paramagnetic center [16,20].

Three alternative techniques have been used to orient biological vesicles: partial dehydration [16,17,20—22,24], strong magnetic fields [18] and viscous flow [19,23]. Each method has its relative advantages and disadvantages. Partial dehydration, particularly in a 90% relative humidity chamber [16,21,22, 24] yields the best orientation in terms of high 'dichroic ratios', but has the disadvantage that it takes 24—36 h for good partial dehydration, and it is difficult to manipulate the ambient potential and pH of the membranes without destroying their orientation. Magnetic alignment yield much lower

'dichroic ratios', but is much quicker and allows greater control of the E_h and pH. Flow techniques have similar advantages and limitations, but in addition require vesicles with a specific elongated shape.

Table I lists the EPR lines which exhibit orientation dependence (Figs. 1, 5 and 7). Simple trigonometry, based on Pythagoras' Theorem, indicates that the sum of the squared cosines of the angles subtended by the three axes of each paramagnetic center from the membrane normal (ϕ) must equal 1.0. We may thus identify which set of g-values belong to the particular paramagnetic centers. Our data demonstrate that the Rieske cluster and both iron-sulfur Clusters A and B have a specific orientation within the chloroplast membrane. Several other paramagnetic centers also show a strong orientation dependence. We will discuss them one by one.

The Rieske cluster

This signal, which is seen most clearly in nonilluminated chloroplasts, has prominent g-values at $g = 1.90$ and $g = 2.03$ (Fig. 1, Ref. 3). The $g = 1.90$ axis lies perpendicular to the membrane plane, while the $g = 2.03$ axis lies in the plane of the membrane [23]. In mitochondria and photosynthetic bacteria, the 'Rieske' $g = 1.90$ cluster has rhombic symmetry, with g-values at 2.03, 1.90 and 1.82 [4,5]. The chloroplast signal only has two clear g-values; this reflects either an axial symmetry, with the two identical axies in the plane of the membrane at $g = 2.03$, or a rhombic symmetry with a very broad, currently unresolved signal at one of the principal g-values. If of rhombic symmetry the missing axis must lie in the plane of the membrane, so we may rule out the signals seen in Fig. 2 at $g = 2.3$ and $g = 1.73$ because both are orthogonal to the membrane. It is appropriate to note that the g_x signal in both mitochondria and bacteria [4,5] is very broad. Alternatively, the saturation characteristics of the g_x component could be different than the g_z and g_y components leading to the nonobservability of the g_x signal. The microwave power and temperature

TABLE I

THE ORIENTATION DEPENDENCE OF THE EPR SIGNALS OF SPINACH CHLOROPLASTS

g-Value	Orientation relative to membrane plane (θ)	Orientation relative to membrane normal (ϕ) (90—θ)	$\cos^2\phi$
Dark signals			
2.3	90	0	1.00
2.03	0	90	0.00
1.90	90	0	1.00
1.73	90	0	1.00
Light-induced signals			
2.15	90	0	1.00
2.07	50	40	0.59
2.05	25	65	0.18
2.04	0	90	0.00
1.94	40	50	0.41
1.92	90	0	1.00
1.89	0	90	0.00
1.86	40	50	0.41

dependence of the Rieske cluster EPR signals could distinguish this latter possibility.

The mitochondrial Rieske cluster is thought to be a two iron, two-sulfur cluster [1,24] and an attempt has been made to correlate the magnetic axes with the chemical structure [24], using a model similar to that proposed for soluble spinach ferredoxin by Gibson et al. [26]. This model, which had received substantial experimental support [27—29] suggests that the g_z principal axis, with the g-value closest to $g = 2.0$, corresponds to the Fe-Fe axis of the binuclear cluster. In mitochondria the g_z signal, at $g = 2.025$, is maximal when the applied magnetic field is parallel to the membrane, as is the g_y signal at $g = 1.90$ [24]. Appropriately, the g_x signal at $g = 1.82$ is maximal when the magnetic field is along the normal to the membrane [24]. Our data suggest that the chloroplast cluster, if it is a binuclear cluster, has a somewhat similar alignment in the membrane. The $g = 2.03$ axis is in the plane of the membrane, but the $g = 1.90$ axis is orthogonal to the membrane plane. If the $g = 2.03$ axis is the Fe-Fe axis, the chloroplast center cannot have axial symmetry, and we must assign our inability to resolve a third g-value to this being a very broad signal. Furthermore, if the Gibson model is appropriate for the chloroplast Rieske center; all four structures whose orientation has been studied (mitochondrial and chloroplast Rieske centers, mitochondria center S-1, and one of the N-1 centers), have the Fe-Fe axis in the plane of the membrane [24].

When DBMIB is added to dark-adapted chloroplasts, the absorption near $g = 1.90$ decreases with a concomitant increase in absorption at $g = 1.95$ (Fig. 3). The remaining $g = 1.90$ signal is still oriented perpendicular to the membrane and the new $g = 1.95$ signal is oriented in the plane of the membrane (Fig. 4). With saturating amounts of DBMIB this effect has been shown to completely abolish the $g = 1.90$ absorption signal with the concomitant generation of the $g = 1.95$ signal [25]. The nature of this effect is not clear, but a further study of the DBMIB effect may give information concerning the inherent symmetry of the Rieske cluster and its relationship with quinones in the electron transport chain of chloroplasts.

Iron-sulfur Clusters A and B

Our discussion of the orientation of these clusters is complicated by the finding of three light-induced signals in the g 2.05 region. It is unlikely that the signal at g 2.07 belongs to the component X [12] because the temperature of observation is too high [12], the microwave power is too low [12], the orientation is not that reported by Dismukes and Sauer [23] and we cannot resolve the signal of X at g 1.76. However, there are many other possible explanations for the three signals. Perhaps the two most likely are that there are either three separate iron-sulfur clusters, A, B, and a new component C, or only two clusters, A and B, with the third signal reflecting an interaction between the two clusters.

In unoriented samples, illumination of dark adapted chloroplasts at cryogenic temperatures reduces Cluster A with almost no contribution from Cluster B [7], so the assignment of the features at g 2.05, 1.94 and 1.86 to the g_z, g_y and g_x axes of Cluster A seems unambiguous. As shown in Table II, the orientation of these three axes are appropriate for them belonging to the same cluster.

The assignment of principal g-values to Cluster B is complicated by the fact that Cluster B is never observed by EPR without a reduced Cluster A signal, at least in spinach chloroplasts. Nevertheless, Cluster B is usually assigned the principal g values g_z 2.05, g_y 1.92, g_x 1.89 [8—10] and indeed Table II shows that the set of g values g_z 2.04, g_y 1.92 and g_x 1.89 satisfies the magnetic axis orientation requirements. However other combinations are equally allowed on the basis of their orientation, and one alternative set stands out; g_z 2.07, g_y 1.94 and g_x 1.89. Cammack et al. [36] have reported similar values for Cluster B in the blue green alga *Phormidium laminosum*.

The decrease of the g 1.86 signal concomitant with the reduction of Cluster B [8—10] is widely attributed to spin-spin interaction between Clusters A and B, and indeed the disappearance of the g 1.96 signal is strong evidence in favor of the suggestion that the three signals in the g 2.05 region belong to Cluster A, Cluster B, and some sort of interacting conglomerate of A and B, rather than to three independent clusters [10]. The disappearance of the g 1.86 signal is usually attributed either to a shift to the g 1.89 region, or to a broadening until it effectively disappears. If the former were the case we might expect to see a component of the g 1.89 signal having an orientation similar to that of the g 1.86 signal, but our data show no such correlation. Unfortunately the orientation of the chloroplast membranes illuminated during freezing (Fig. 7) was apparently too poor to resolve the individual features of the g 2.05 region (cf. Fig. 5) so we cannot rule out a change in the orientation of Cluster A to bring the g 1.86 axis co-planar with the g 1.89 axis of Cluster B. Nevertheless, the orientation of the g 1.94 axis does not seem to change significantly when Cluster B is reduced (cf. Figs. 6 and 8).

The recognition of three signals in the g 2.05 region, and the two alternative possible sets of g values for Cluster B, suggest that there are other changes concomitant with the disappearance of the g 1.86 signal attributed to Cluster A. For example, if Cluster B has g values at 2.04, 1.92 and 1.89, the signal at g 2.07 may appear as the g 1.86 signal disappears. Alternatively if Cluster B has

TABLE II

ASSIGNMENT OF g-VALUES AND ORIENTATION TO THE IRON-SULFUR CLUSTERS OF SPINACH CHLOROPLASTS

For definition of θ and ϕ see Table I.

Fe-S centers	g-value	θ	ϕ	$\cos^2\phi$	$\Sigma\cos^2\phi$
Center A	g_z 2.05	25	65	0.18	
	g_y 1.94	40	50	0.41	1.00
	g_x 1.86	40	50	0.41	
Rieske center	g_z 2.03	0	90	0	
	g_y 1.90	90	0	1	1.00
	g_x ?	0 (deduced)	90	0	
Center B	g_z 2.04	0	90	0	
	g_y 1.92	90	0	1	1.00
	g_x 1.89	0	90	0	
Center B	g_z 2.07	50	40	0.59	
	g_y 1.94	40	50	0.41	1.00
	g_x 1.89	0	90	0	

the g values 2.07, 1.94 and 1.89, the signals at g 2.04 and 1.92 may appear as the g 1.86 signal disappears. In either case the new signals could be attributed to the interaction of Clusters A and B. Such an assignment could be tested by EPR at higher frequencies.

Unfortunately, until we have far more information about the physical nature of the interactions between the iron-sulfur clusters, computer simulation of the oriented spectra [22] will be impossible, and our conclusions will have to remain rather tentative. Nevertheless, it is clear that both Clusters A and B show a high degree of orientation, for example the g 1.94 axis shows a dichroic ratio of more than 7. These results are in apparent contradiction with those of Dismukes and Sauer [23] who reported no orientation of Cluster A. However their work only examined the membranes with applied magnetic fields perpendicular to, or parallel with, the oriented membranes. Since both the g = 1.94 and g = 1.86 axes are aligned at 40° from the membrane plane, spectra recorded at 0° and 90° from the plane show little difference (Fig. 5). Our data agree with Dismukes and Sauer in that the g = 1.92 axis, normally assigned to Cluster B, is orthogonal to the membrane.

Both iron-sulfur Clusters A and B are thought to be tetranuclear iron sulfur cluster [30]. Unfortunately, no model for such clusters [31—35] has been developed as fully as the Gibson model for the binuclear types, and we are unable to assign structural axes to the magnetic axes measured here. A summary of the orientations of the magnetic axis of the iron sulfur centers within the chloroplast membrane, including the two possible assignments of Cluster B, is shown in Fig. 9.

Other paramagnetic centers

The dark-adapted chloroplasts exhibited two other prominent, rather broad, EPR signals in the iron-sulfur region of the spectra at g = 2.3 and g = 1.73. Both are oriented orthogonal to the membrane plane so the simplest explanation is

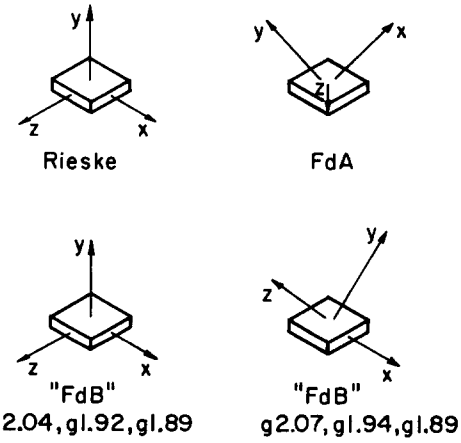

Fig. 9. Isometric projections of the magnetic axes of the iron-sulfur clusters in the chloroplast membrane. Rieske Cluster, g_z = 2.03, g_y = 1.90 and g_x undetectable in our experiments: but deduced to be oriented as shown; Cluster A, g_z = 2.05, g_y = 1.94, and g_x = 1.86; Cluster B, two possible orientations of this center depending upon g-value assignments.

that they belong to two different paramagnetic centers. These signals have not been reported before, presumably bacause they are too broad in unoriented samples. Their spectrum does not change upon illumination either at room temperature or at 15 K. The functions of the species which these signals represent are unknown.

In contrast, the signal at $g = 2.15$, oriented orthogonally to the membrane plane is generated during illumination at low temperatures. This signal is rather broad, and again has not been reported previously. Since it is generated by light at low temperatures it is tempting to assign it to some paramagnetic center associated with one of the two photosystems, but more work will be required to clarify this point. In view of the orientation of this signal, it seems unlikely that it is associated with the interaction of Clusters A and B.

The orientation studies offer the distinct advantage of concentrating spins for improved EPR observability. This is evidenced by the increased content of Cluster B observable in the low temperature illumination experiments and the new signals reported at $g = 2.15$, 1.73, and 2.3.

Acknowledgments

We would like to thank Haywood Blum, Jack Leigh, Harry Frank, Bob Goldstein, Chris Moser and Richard Cammack for helpful discussions. We would also like to thank Richard Malkin for the DBMIB. This work is supported through research grants from the National Science Foundation (Biophysics Research Program) and the Department of Energy through the Division of Biology and Medicine of the Lawrence Berkeley Laboratory.

References

1 Ohnishi, T. (1979) in Membrane Proteins in Energy Transduction (Capaldi, R.A., ed.), pp. 1—87, Marcel Dekker, New York
2 Malkin, R. and Bearden, A.J. (1978) Biochim. Biophys. Acta 505, 147—181
3 Malkin, R. and Aparicio, P.J. (1975) Biochem. Biophys. Res. Commun. 63, 1157—1160
4 Rieske, J.S., Zaugg, W.S. and Hansen, R.E. (1964) J. Biol. Chem. 239, 3023—3030
5 Prince, R.C., Lindsay, J.G. and Dutton, P.L. (1975) FEBS Lett. 51, 108—111
6 Malkin, R. and Posner, H.B. (1978) Biochim. Biophys. Acta 501, 552—554
7 Malkin, R. and Bearden, A.J. (1971) Proc. Natl. Acad. Sci. U.S.A. 68, 16—19
8 Evans, M.C.W., Telfer, A. and Lord, A.V. (1972) Biochim. Biophys. Acta 267, 530—537
9 Ke, B., Hansen, R.E. and Beinert, H. (1973) Proc. Natl. Acad. U.S.A. 70, 2941—2945
10 Evans, M.C.W., Reeves, S.G. and Cammack, R. (1974) FEBS Lett. 49, 111—114
11 McIntosh, A.R., Chu, M. and Bolton, J.R. (1975) Biochim. Biophys. Acta 376, 308—314
12 Evans, M.C.W., Sihra, C.K. and Cammack, R. (1976) Biochem. J. 158, 71—77
13 Evans, E.H., Cammack, R. and Evans, M.C.W. (1976) Biochem. Biophys. Res. Commun. 68, 1212—1218
14 McIntosh, A.R. and Bolton, J.R. (1976) Biochim. Biophys. Acta 430, 555—559
15 Malkin, R. (1971) Biochim. Biophys. Acta 253, 421—427
16 Blum, H., Harmon, H.J., Leigh, J.S., Salerno, J.C. and Chance, B. (1978) Biochim. Biophys. Acta 502, 1—10
17 Hales, B.J. and Das Gupta, A. (1979) Biochim. Biophys. Acta 548, 276—286
18 Geacintov, N.E., van Nostrand, F., Becker, J.F. and Tinkel, J.B. (1972) Biochim. Biophys. Acta 267, 65—79
19 Dismukes, G.C., McGuire, A., Blankenship, R .E. and Sauer, K. (1978) Biophys. J. 21, 239—256
20 Blasie, J.K., Erecinska, M., Samuels, S. and Leigh, J.S. (1978) Biochim. Biophys. Acta 501, 33—52
21 Tiede, D.M., Leigh, J.S. and Dutton, P.L. (1978) Biochim. Biophys. Acta 503, 524—544
22 Blum, H., Salerno, J.C. and Leigh, J.S. (1978) J. Mag. Reson. 30, 385—391

23 Dismukes, G.C. and Sauer, K. (1978) Biochim. Biophys. Acta 504, 431—445
24 Salerno, J.C., Blum, H. and Ohnishi, T. (1979) Biochim. Biophys. Acta 547, 270—281
25 Chain, R.K. and Malkin, R. (1979) Arch. Biochem. Biophys. 197, 52—56
26 Gibson, J.F., Hall, D.O., Thornley, J.F. and Whatley, F. (1966) Proc. Natl. Acad. Sci. U.S.A. 56, 987—990
27 Johnson, C.E., Cammack, R.E., Rao, K.K. and Hall, D.O. (1971) Biochem. Biophys. Res. Commun. 43, 564—571
28 Sands, R.H. and Dunham, W.R. (1975) Q. Rev. Biophys. 7, 443—504
29 Blum, H., Leigh, J.S., Ohnishi, T. and Salerno, J.C. (1978) in Frontiers of Biological Energetics Dutton, P.L., Leigh, J.S. and Scarpa, A., eds.), pp. 625—633, Academic Press, New York
30 Cammack, R. and Evans, M.C.W. (1975) Biochem. Biophys. Res. Commun. 67, 544—549
31 Yang, C.Y., Johnson, K.H., Holm, R.H. and Norman, J.G. (1975) J. Am. Chem. Soc. 97, 6596—6598
32 Thomson, A.J. 91975) Biochem. Soc. Trans. 3, 468—472
33 Holm, R.H. and Ibers, J.A. (1977) in Iron Sulfur Proteins, III (Lovenberg, W., ed.), pp. 206—279, Academic Press, New York
34 Blum, H., Salerno, J.C., Prince, R.C., Leigh, J.S. and Ohnishi, T. (1977) Biophys. J. 20, 23—31
35 Blum, H., Salerno, J.C. and Cusanovich, M.A. (1978) Biochem. Biophys. Res. Commun. 84, 1125—1128
36 Cammack, R., Dyan, M.D. and Stewart, A.C. (1979) FEBS Lett. 107, 422—426