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Orientation of EPR signals arising from components in Photosystem II membranes

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EPR signals arising from components in oriented multilayers of Photosystem II (PS II) membranes have been studied and the following results have been obtained. (1) The EPR signals arising from the primary semiquinone-iron complex (Q_A^-Fe) were highly oriented, with features at $g = 1.90$, $g = 1.82$ and $g = 1.66$ showing maxima when the membranes were perpendicular to the magnetic field. (2) The EPR signal, arising from the reduced pheophytin acceptor interacting with Q_A^-Fe, showed an orientation-dependent splitting, ranging from 39 G when the membranes were parallel to the magnetic field to 27 G when the membranes were perpendicular to the magnetic field. (3) The S_2 multiline signal associated with the O_2-evolving enzyme showed an orientation dependence. This was most marked as position shifts in the low-field wings of the spectrum. These effects indicate that the component is oriented within the membrane and has some magnetically anisotropic character. (4) The component at $g \simeq 4$, thought to be due to an oxidized charge carrier close to S_2, showed a slight orientation dependence in its amplitude, but a significant orientation-dependent field-position shift was present, indicating that this is a magnetically anisotropic centre with a fixed geometry in the membrane. (5) Cytochrome *b*-559 in its oxidized form showed large highly oriented signals. The g_z 2.97 feature was maximum when the membranes were oriented parallel to the magnetic field, while the g_y 2.22 was maximum when the membrane plane was perpendicular to the magnetic field. This indicates that the haem plane is perpendicular to the plane of the membrane, in agreement with previous reports using chloroplasts. Ageing of the sample brings about a change from low- to high-spin state accompanied by a change in orientation of the haem relative to the membrane (from perpendicular to approximately 45°). (6) Signal II slow, which is present in the dark and which arises from a component which acts as an electron donor in PS II, is highly oriented. The signal becomes resolved into two different symmetrical four-line spectra. When the membranes were parallel to the magnetic field a narrow signal centred at $g \approx 2.0032$ was present, while when the membranes were perpendicular a wider signal centred at $g \approx 2.0061$ was present. The *g*-shift may be taken as an indication that the semiquinone ring is perpendicular to the membrane. (7) The spin-polarized triplet state of P-680, the primary donor chlorophyll, can be photoinduced in oriented PS II multilayers. The *Z* transition was maximum when the membranes were oriented perpendicular to the magnetic field, while the *X* and *Y* transitions were maximum when the membranes were parallel to the magnetic field. This indicates that the plane of the chorophyll ring is parallel to the plane of the membrane.

Introduction

Quite extensive orientation studies have been carried out on EPR signals observed in Photosys-

Abbreviations: DCMU: 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Mes, 4-morpholineethanesulphonic acid; Chl, chlorophyll; Ph, pheophytin

tem I (PS I) [1–4] and purple bacteria [5–8]. The results in bacteria, taken together with measurements of linear and circular dichroism (reviewed in Ref. 9) and magnetophotoselection (i.e., Ref. 10) have allowed models of the reaction centre to be proposed in which the geometry of some of the components has been established (reviewed in Ref. 11).

Despite the discovery of several EPR signals arising from Photosystem II (PS II) components over the last few years (reviewed in Ref. 12), few orientation studies of EPR signals from this photosystem have been reported [13–19].

In this work, a systematic study of the EPR signals present in oriented multilayers of PS II is reported.

Methods

PS II membranes were prepared from market spinach using the method of Berthold et al. [20] with the modifications used in Refs. 21 or 22. The membranes were resuspended at high concentration (approx. 8 mg Chl/ml) in either buffer 1 (400 mM sucrose/20 mM Mes (pH 6.0)/15 mM NaCl/ 5 mM $MgCl_2$) or in a buffer comprising 5 mM Mes (pH 6.0)/5 mM $MgCl_2$ (see legends to figures). 1 mM EDTA was added to the resuspension medium or to the already dried films. The suspension of membranes was painted onto mylar sheets and dried in a 90% humidity atmosphere in darkness at 4°C for 12–36 h (see Ref. 72). Two to six sheets were placed into quartz EPR tubes, the tubes were flushed with helium gas and then sealed. The samples were cooled to liquid helium temperature and EPR spectra were taken over a series of orientations relative to the magnetic field of the EPR machine. In some cases, samples were cooled and stored in liquid nitrogen in darkness before EPR spectra were taken.

Chemical reduction of oriented samples was carried out by adding enough fresh sodium dithionite solution (20% in 200 mM glycine-KOH buffer (pH 9.0)) to an EPR tube so that the membrane multilayers were submerged. The reduced samples were incubated at room temperature in darkness for 7.5 minutes under O_2-free argon gas before being frozen. This treatment had little effect on the orientation of the membranes, since residual signal II present in the reduced membranes remained highly oriented.

Illumination of samples in the EPR cavity was carried out using an 800 W projector and perspex light guide. Illumination at 77 K and at 200 K was carried out using the same projector, but samples were submerged in a bath containing liquid N_2 or a solid CO_2/ethanol mixture.

Results and Discussion

The electron acceptor side of PS II

The semiquinone-iron complex

The primary quinone acceptor of PS II [23] is associated with an iron atom [24,25]. The semiquinone-iron complex gives rise to two different EPR signals; a signal at $g = 1.82$ [26,27] and a signal at $g = 1.90$ which is favoured at neutral and high pH [28,29]. Both of these signals also exhibit features at higher field ($g = 1.66$) [21,27–29]. Signals very similar to those in PS II had been found and characterised earlier in purple bacteria (reviewed in Refs. 30, 31).

The semiquinone-iron signals in PS II are stably photoreduced if illumination of PS II is given at low temperature. Fig. 1a shows that this is also the case when oriented multilayers of PS II membranes are illuminated at 77 K. The intensities of the semiquinone-iron signals are greatly enhanced and very obvious orientation effects can be observed. Features at $g = 1.90$, $g = 1.82$ and $g = 1.66$ all show marked maxima when the membrane surface is oriented perpendicular (90°) to the magnetic field. When the membrane surface is parallel (0°) to the magnetic field, the $g = 1.90$ signal is almost absent; the $g = 1.82$ is diminished but is present as a step rather than a spike. The $g = 1.66$ signal is almost absent, but a new but much smaller high-field feature is now observable at $g \simeq 1.69$. The shape and width differences of the signal present at 0° might be due to some kind of heterogeneity of centres. Whether this is due to an inherent heterogeneity of PS II or to an artifact induced by drying is not known.

Polar plots of the semiquinone iron signal showed that the $g = 1.90$, $g = 1.82$ and $g = 1.66$ features are all oriented perpendicular to the membrane surface (not shown).

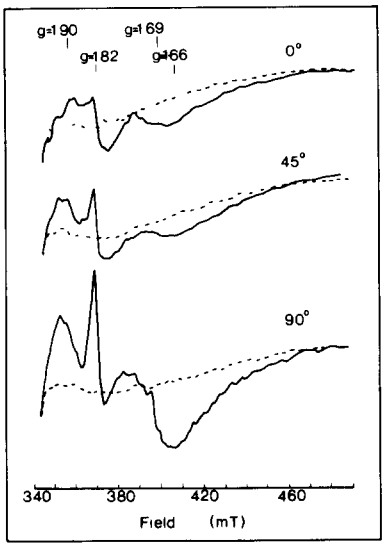


Fig. 1. Light-induced Q_A^-Fe (solid lines) in oriented PS II membranes. The broken lines were recorded in samples prior to illumination at 77 K (10 minutes). The angles marked in the figure indicate the orientations of the plane of the membrane relative to the measuring magnetic field. Freshly made PS II membranes were resuspended in 5 mM $MgCl_2$/5 mM Mes (pH 6.0)/1 mM EDTA and dried on mylar in darkness for 12 h in a 90% humidity atmosphere at 4°C. EPR conditions were as follows: microwave power, 35 mW; modulation amplitude, 20 G; temperature, 4.8 K; microwave frequency, 9.45 GHz; gain, $1.25 \cdot 10^5$, scan speed 25 G per s, response time 200 ms. Two sheets of mylar were used

The semiquinone-iron signals were present in darkness when oriented multilayers of PS II membranes were soaked in a sodium dithionite solution for 7.5 min. The same marked oriented features were observed in the chemically reduced centres as were observed in those photochemically reduced (not shown).

In most experiments, illumination at 77 K also resulted in the photo-induction of an EPR signal at $g \simeq 2.045$ (not shown). This signal had very little orientation dependence. The signal disappeared when the sample was warmed to room temperature, incubated in darkness for 2 min then refrozen, whereas the Q_A^-Fe signal was dimished by only 50% by this treatment. This signal is observed in non-oriented membranes and is affected by bicarbonate depletion [32]. If this is an acceptor (on a side path or otherwise) it would appear to be less stable and thus perhaps more primary than Q_A^-Fe.

In bacteria, orientation of the analogous Q_A^-Fe signals has been observed in a number of species [7,8]. In Ref. 7 the $g = 1.82$ signal was maximum when the membrane was at 90° to the magnetic field, just as observed here in PS II. However, in *Rhodopseudomonas sphaeroides* and *Chromatium vinosum* the high-field feature at around $g = 1.68$ was small and oriented orthogonally to the $g = 1.82$ feature [7].

Two recent papers have attempted theoretical explanations of the semiquinone-iron interactions responsible for the EPR signal present in photosynthetic bacteria [8,34]. Both models based on the spin Hamiltonian formalism envisage a high-spin Fe^{2+} atom in a low symmetry [8] or asymmetric [34] crystal field which splits the ground manifold into five magnetic sublevels. Each sublevel interacts with semiquinone to form a doublet. In the model of Dismukes et al. [8] an isotropic exchange interaction was assumed and using the parameters previously obtained from a magnetic susceptibility study [73] together with the orientation data from *Rps. viridis*, a model was put forward which explained the Q_A^-Fe signal as arising from the three lowest-lying doublets and a spectrum was simulated [8]. A dipolar term had to be included to explain to broadening of the $g = 1.7$ feature and from the magnitude of this term the distance between the iron and the semiquinone was calculated to be 0.62–0.78 nm [8]. However, as pointed out in that work [8], a major drawback of this model is that the $g = 1.7$ feature results from the first excited state doublet and thus would be predicted to be decreased as the temperature is lowered. This is not found to be the case. In fact, lowering the temperature enhances the $g = 1.7$ feature [34]. Butler et al. [34] have put forward an alternative model which explains the EPR spectrum as being due to only the two lowest-lying doublets. The interaction between the iron and the semiquinone is antiferromagnetic [73] and is anisotropic, but the relative contributions of anisotropic super exchange and dipolar interactions has not been determined. Good matching of the theoretical spectrum to experimental data was demonstrated with variations in temperature, microwave frequency and preparation conditions. In this model the wide absorption at $g = 1.8$ (observed as the $g = 1.82$ and $g = 1.7$ features in derivative spectra) arises

from transitions along the x and z axis of the magnetic interaction tensor of the two doublets [34]. Unfortunately, no orientation data were reported and previously reported orientation data were not discussed [6,7]. It is difficult to reconcile such a model with the orientation data reported here, since all of the EPR features around $g = 1.8$ show marked maxima when the membranes are perpendicular to the magnetic field and only a single axis can be maximum at this orientation in a two-dimensionally ordered system. It may be possible to improve the fit of the model to the experimental data in Ref. 34 by introducing anisotropic linewidths, and this could reconcile the model with the orientation data. However, the PS II data are further complicated by the existence of the $g = 1.9$ EPR form which has not been considered in the model of Butler at al. [34]. Thus interpretation of the orientation-dependence of the semiquinone iron signal in PS II must await further advances in our understanding of the analogous signals in bacteria. Such advances could occur in the near future when EPR measurements are carried out on crystals of bacterial reaction centres [38,74].

The results obtained here demonstrate that the semiquinone-iron complex is oriented in a fixed conformation in the membrane and that its EPR signal shows an orientation dependence similar to that observed in purple bacteria.

The split pheophytin$^-$ signal

When the semiquinone-iron complex is chemically reduced illumination at low temperature results in formation of a large split signal attributed to the reduced pheophytin acceptor interacting with the semiquinone-iron complex [24,26,27,35] by analogy to the situation in bacteria [36,37]. Fig. 2 shows that the split Ph^- signal can be photoinduced by 200 K illumination of oriented multilayers of PS II membranes that had been frozen in a sodium dithionite solution. A marked orientation dependence of the signal shape is observed. The presence of Signal II in the dark (not shown) indicates that the sample is only partially reduced, and thus the split Ph^- signal observed probably represents only that proportion of centres in which Q_A^-Fe is chemically reduced (see Ref. 27).

The orientation dependence of the split Ph^- signal is manifest as a splitting difference rather

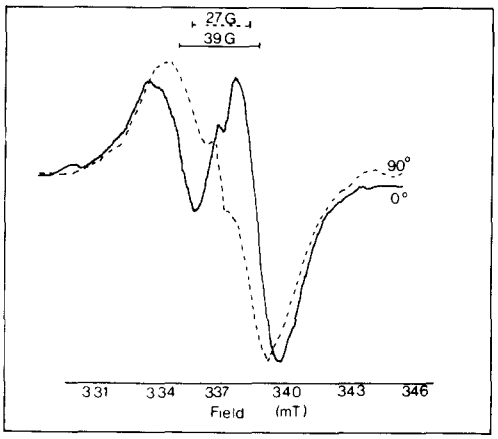


Fig. 2. The split Ph^- signal in oriented PS II membranes was induced by a period of 4 min of illumination at 200 K in a sample reduced by dithionite. Sample preparation was described in Fig. 1 except that the multilayers were submerged in a 20% sodium dithionite solution (in 200 mM glycine (pH 9.0)) for 7.5 min in darkness, under O_2-free argon before freezing. EPR conditions were as follows: microwave power, 2 dB down from 200 mW; modulation amplitude, 6.3 G; temperature, 4.8 K; frequency, 9.45 GHz; gain, $1.25 \cdot 10^5$. The high microwave power was used to diminish the relative contribution of the $g \approx 2.00$ radical present in the dark. The data shown are light-minus-dark spectra.

than a change in amplitude. The splitting of the signal when the membrane surface was parallel to the magnetic field was approx. 39 G, while that when the membrane surface was perpendicular was less than 27 G (Fig. 2). A polar plot of the splitting clearly showed the maximum splitting at 0°C (not shown).

It is of note that the splitting of the split Ph^- has been reported to vary depending on pH [29] and the presence of herbicides [21]. However, the previously reported splitting values range from 55 G (pH 8.5) to 38 G (pH 6.0 or in the presence of 1-dinoseb) in non-oriented samples [21,29]. These two extremes are thought to reflect the interaction with the $g = 1.90$ and the $g = 1.82$ EPR forms of the Q_A^-Fe signal, respectively [29]. Since the samples are only partially reduced, the observed small splitting could arise from a preferential reduction of the centres which have the $g = 1.82$ form of the semiquinone iron complex. At present, however, there is no evidence that the $g = 1.9$ and $g = 1.82$ signals represent acceptors with different thermodynamic properties.

Detailed analysis of the data is premature at this time, since the possible existence of heterogenity in PS II giving rise to differently oriented populations of Q_A^-Fe could have a large influence on the phenomenology.

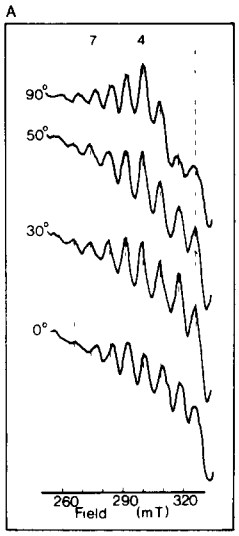
Due to the poor understanding of the magnetic interactions responsible for the split signal, the orientation data provide little information on the orientation of the Ph^- itself, although it is clear that the geometry between Ph^- and the Q_A^-Fe is fixed in the membrane. It is of note that optical work has shown that the Q_Y transition of Ph (and thus the plane of its macrocycle) is oriented perpendicular to the membrane [39,40].

The donor side

The S_2 multiline signal

When PS II is given a flash at room temperature [41], frozen under illumination [42] or illuminated at 200 K [43], an EPR signal is formed which is attributed to S_2 [41–44]. The signal is thought to arise from oxidation of a manganese complex which is involved in the O_2-evolving process. Fig. 3 shows the multiline S_2 signal that was formed in oriented multilayers of PS II membranes after illumination was given at 200 K. The major differences between the two spectra recorded under these conditions at 0° and 90° can be attributed to oriented semiquinone-iron acceptor at high field (not shown) which undergoes photoreduction under these conditions and oriented cytochrome *b*-559 at low field which is mostly oxidized in the dark but which may contribute to the light-induced signal depending on the redox state and intactness of the preparation. Apart from these differences, it is of note that lowest-field peaks of the multiline signal are more easily discerned in the spectrum recorded when the membranes were perpendicular to the membrane.

Fig. 3 also shows a comparison of the multiline S_2 signal recorded at 50° and 30° with those recorded at 0° and 90°. Although the position of the large peaks close to $g = 2$ remains unchanged, the outer peaks (peaks 7 and 8 downfield from $g = 2.0$) show marked and repeatable position changes. This is plotted in Fig. 3b and it can be seen that a shift to low field occurs with a maximum at approximately 45°.



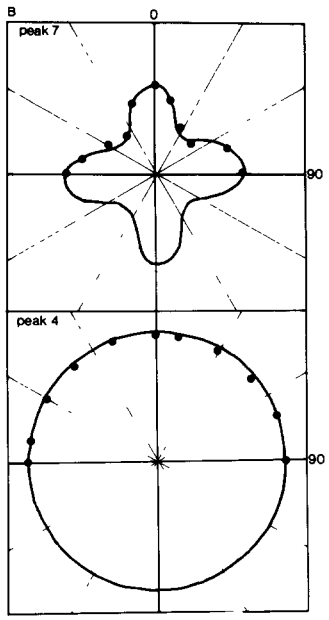


Fig. 3. Orientation-dependent field shifts in the wings of the multiline S_2 signal. The signal was induced by 2 min of illumination at 200 K. EPR conditions were as in Fig. 1 except that the temperature was 8 K. The samples were suspended in 0.4 M sucrose/15 mM sodium chloride/20 mM Mes (pH 6.0)/5 mM $MgCl_2$ before drying for 12 h. (A) Spectra were recorded with the membranes at 90°, 50°, 30° and 0° to the magnetic field. (B) Polar plots of this effect, upper plot is of the 7th peak counting from $g = 2$ towards low field, while the lower plot is the 4th peak counting the same way. The plots are of the field position relative to fixed arbitrary reference field values downfield from the peak in question.

The observation of orientation dependence of the S_2 signal amplitude made here is in contradiction with a previous report by Hansson et al. [19] in which chloroplasts were frozen under illumination in a magnetic field. It was concluded that the component giving rise to the multiline signal was probably oriented in the membrane but was magnetically isotropic [19]. The orientation effects observed here clearly demonstrate that the component is oriented and show the presence of some anisotropic character. The anisotropy of the multiline S_2 signal reported here should be influential in future attempts to understand the types of interaction responsible for this signal.

The $g \approx 4$ component

Recently, EPR signals at $g \approx 4$ have been discovered which have been attributed to a charge carrier close to the S states [44,46]. At 200 K a signal can be photoinduced which is thought to arise from a component acting as a carrier closer to the reaction centre than S_2. The signal is formed in the presence of S_2 and is thought to represent a pre-S_3 state [44]. When illumination is given at lower temperature, a $g \approx 4$ signal is formed as a precursor to S_2 [46]. It has beensuggested that this signal represents a donor to the component responsible for Signal II_{vf} and the acceptor of electrons from S_1 and S_2 [44].

In dried oriented multilayers, illumination of samples at 200 K gives rise to a light-induced signal at $g \approx 4.1$ which has a linewidth approximately similar to that reported earlier (360 mT) [44,46] (Fig. 4).

An orientation-dependent field shift and amplitude change are present in the light-induced signal, the signal at 0° being smaller and at lower field than the signal at 90°. This shows that the component is oriented in the membrane and is anisotropic.

The chemical origin of the $g = 4.1$ signal is unknown. Its suggested origins include rhombic ferric iron [46,44] and manganese [44]. The orientation characteristics observed here may help to distinguish between some of the possible candidates.

Cytochrome b-559

Cytochrome *b*-559 can be clearly observed by

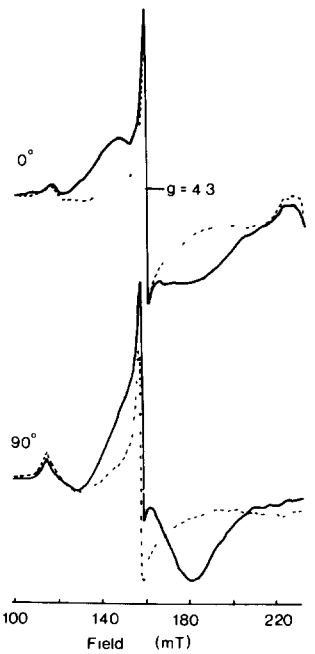


Fig 4 The light-induced $g \approx 4$ component in oriented PS II membranes The dotted lines were recorded in darkness, while the solid lines were recorded after 4 min of illumination at 200 K EPR and sample conditions were as described in Fig 3 except that the sample (in 5 mM $MgCl_2$ and 5 mM Mes (pH 6 0)) was dried under a 90% humidity argon atmosphere for 12 h

EPR in PS II membranes [21]. The EPR signals arising from low-spin ferric haem, discovered by Malkin and Vänngård [47] (see also Ref. 48) have been quite extensively studied in oriented chloroplasts [4,14,15]. The amplitude of the g_z ($g = 3.08$ high potential, $g = 2.94$ low potential) is maximum when the membranes are oriented at 90° to the magnetic field, while the g_y ($g = 2.16$ high potential, $g = 2.26$ low potential) is maximum when the membranes are oriented parallel to the magnetic field [4,14,15].

In multilayers of PS II membranes the drying procedures result in nearly all of the cytochrome *b*-559 being in the oxidized form (the *g* values indicate that both high- and low-potential forms are present) in the dark. The signals are highly orientation-dependent and have maxima exactly as reported earlier for chloroplasts [4,14,15]. The oriented cytochrome *b*-559 signals were routinely used as a measure of orientation in a particular preparation (Fig. 5).

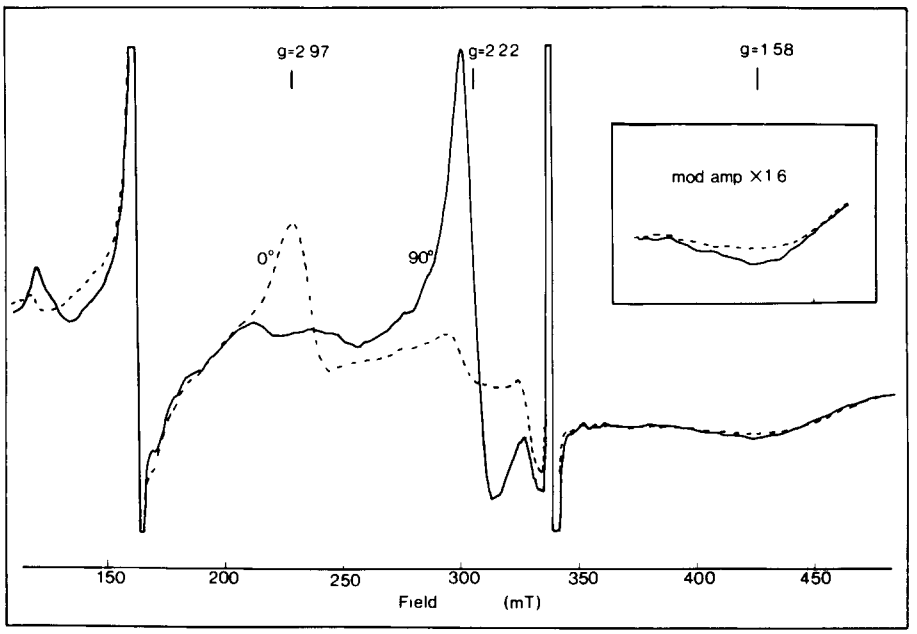


Fig 5 The oxidized cytochrome *b*-559 signal present in the dark in oriented PS II membranes Samples were prepared as in Fig. 1, but the drying time was 48 h EPR conditions were as follows temperature, 20 K, microwave power, 15 dB down from 200 mW; modulation amplitude, 20 G (inset, 32 G), frequency, 9.46 GHz The broken line was recorded when the mebranes were parallel to the magnetic field, while the solid line was recorded with the membranes perpendicular to the magnetic field

In isolated cytochrome *b*-559, the g_x line has been observed at $g \approx 1.54$ [49]. In the oriented membranes used here a small signal at $g = 1.58$ is present. Since in a two-dimensionally-oriented system not more than one *g*-axis can be maximum at 90°, the $g = 1.58$ feature cannot simply be attributed to the g_x component of cytochrome *b*-599.

The EPR orientation results in chloroplasts have previously been used to determine that the cytochrome haem plane is perpendicular to the membrane surface [4,14,15], in agreement with earlier linear dichroism studies [50]. The results reported here confirm this orientation of the cytochrome in isolated PS II membranes.

$g \approx 6$ signals

High-spin ferric haem signals at $g \approx 6.0$ have been attributed to partially denatured cytochromes in chloroplasts [14,15,51]. In PS II membranes only cytochrome *b*-559 is thought to be present (no other cytochromes have been observed optically or by EPR in such preparations). Thus the $g \approx 6.0$ signals observed in such preparation presumably arise from partially denatured cytochrome *b*-559.

In Fig. 6 the $g \approx 6.0$ signals present in oriented multilayers of PS II are shown. Fig. 6 is from a sample in which the S_2 multiline could be photoinduced (i.e., relatively undamaged), while Fig. 6B is from a sample in which no S_2 could be observed

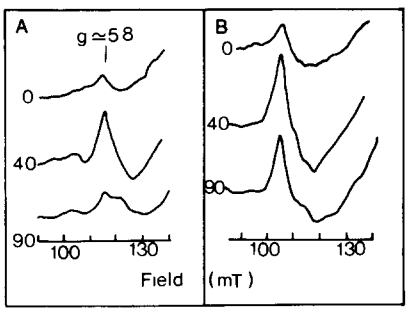


Fig. 6 EPR signals close to $g \approx 6.0$ recorded in oriented PS II membranes (A) Recorded using samples and EPR conditions as in Fig 3; (B) recorded using samples and conditions described in Fig 3 except that drying was carried out for 72 h and the spectra were obtained at 4 5 K

(i.e., less intact). Larger $g \approx 6.00$ signals are present in the less intact preparation. This agrees with the idea that the presence of the $g = 6$ signals reflects denaturation of cytochrome *b*-559. In both cases, a number of signals with slightly different field positions are observed. All of the signals show marked orientation-dependence. The largest signals have maxima at around 45° in both kinds of preparation (Fig. 6), while the small higher-field signal (seen as a shoulder in Fig. 6A, 90°) seems to be maximum when the membrane surface is oriented at 90° to the magnetic field.

For a high-spin ferric haem, the $g \approx 6$ axes are in the plane of the haem [52]. This indicates the small higher-field signal seems to arise from a high-spin haem with the same orientation as that of native low-spin cytochrome *b*-559. However, the larger signals arise from haems oriented differently to low-spin cytochrome *b*-559. Thus the denaturation which gives rise to the spin change also seems to affect the cytochrome's orientation in the membrane.

Signal II

Signal II arises from kinetically different components functioning as donors in PS II [53–58]. One of these components, Signal II_{vf}, is thought to be the donor to P-680 [54]. Another signal, Signal II_s, which is spectroscopically almost identical to Signal II_{vf}, is normally mostly oxidised in the dark but acts as a donor when reduced before illumination [57,58]. The chemical nature of the component is unknown, but it has recently been suggested that it is a semiquinone cation [59].

The orientation-dependence of Signal II_s has been investigated by a number of different groups [13,16–18]. However, the improved dichroic ratios obtained in the present work produce remarkably well-defined orientation effects in Signal II. Fig. 7 shows that Signal II resolves into two apparently different, symmetrical, four-line signals. When the membranes are parallel to the magnetic field, a narrow signal centred at $g \approx 2.0032$ is observed. When the membranes are perpendicular to the magnetic field, a wider signal centred at $g \approx 2.0061$ is observed. It can also be seen that none of the features is common to the two signals.

Recently, similar orientation properties for Signal II have been briefly described [18]. This was

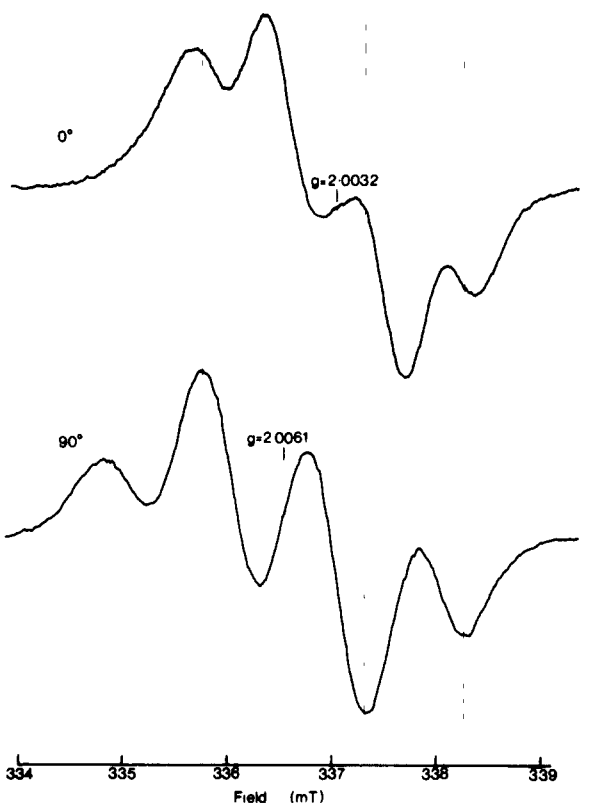


Fig 7 Signal II present in the dark in oriented PS II membranes The sample was prepared as in Fig 6B EPR conditions were as follows: temperature, 20 K; modulation amplitude, 2 G, microwave power (0.05 mW), 35 dB down from 200 mW. Upper trace, membranes parallel to magnetic field, lower trace, membranes perpendicular to the magnetic field Broken lines are to emphasise peak position differences in the two spectra

interpreted in line with the hypothesis that the signal arises from a semiquinone cation [59]. The orientation data were taken as an indication that the partially resolved hyperfine structure of Signal II is due to hyperfine interaction with a single methyl group on the plastoquinone cation radical. It was also suggested that the $C-CH_3$ bond direction and the aromatic ring plane lie perpendicular to the membrane plane [59]. If the orientation-dependent shift reported in this work is largely due to *g* anisotropy, then it may be taken as an indication that the semiquinone ring is perpendicular to the membrane. Semiquinone anions show an EPR absorption close to the free electron value for a direction perpendicular to the aromatic ring plane (the g_z axis), while the directions in the

plane of the ring have higher g values (the g_x and g_y axis [71]. This may also be expected in semiquinone cations. In Fig. 7, the lower g value was obtained when the magnetic field was parallel to the plane of the membrane, suggesting that the g_z axis (as well as one of the other g axes) lies in this plane. When the magnetic field is perpendicular to the membrane plane the higher g value obtained probably reflects uniquely either the g_x or g_y axis. Similar results were interpreted in this way for the primary semiquinone anion in purple bacterial reaction centres [7]. This interpretation is an oversimplication, since the hyperfine anisotropy and the g values of the g axes of Signal II are probably not the same as those obtained for semiquinone in vitro [71].

P-680 triplet

When the semiquinone-iron acceptor is reduced, illumination of PS II at low temperature results in formation of a triplet state of P-680, ^{3}P-680, which is formed by recombination of the P-680 $^+$Ph$^-$ radical pair [60–63]. The characterization of this signal has relied on the analogy with

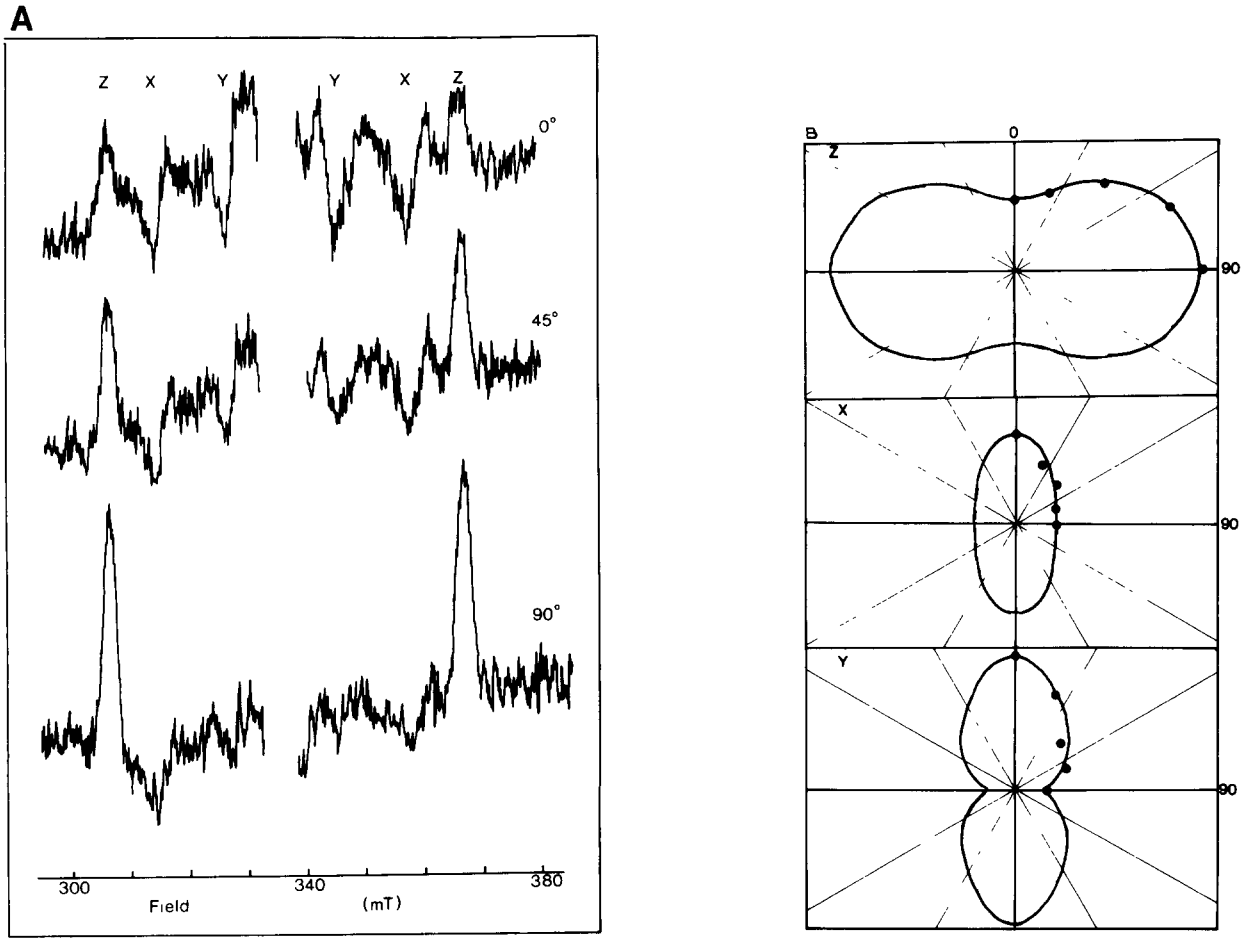


Fig. 8 The P-680 spin-polarized triplet EPR signal photoinduced in oriented PS II particles The PS II membranes were prepared and were reduced by sodium dithionite as described in Fig 2 (A) Spectra were recorded under illumination using the following instrument settings: temperature, 4 2 K; microwave power, 50 dB down from 200 mW; modulation amplitude, 32 G The spectra are an average of 32 scans from which the spectra recorded in the dark have been subtracted (B) Polar plots of the *ZXY* features of the triplet signal. The signal amplitude at high and low field were added together Solid circles show data ponts. Data at 22 5° and 67 5° were obtained from spectra which were the average of 16 scans

the work done on a similar signal observed in purple photosynthetic bacteria [64,65]. The orientation of the triplet EPR signal in bacteria has been studied by a number of groups [5–7]. Hales and Gupta [6] and Tiede and Dutton [7] obtained almost identical results in *Rhodospirillum rubrum* and *Rhodopseudomonas sphaeroides*, respectively. Both groups found that the Z axis was 10–20° away from being parallel to the membrane surface, the Y axis was 10–20° from being perpendicular to the membrane surface and the X axis was parallel to the membrane surface. In bacteria it is accepted that the primary donor is a special pair of bacteriochlorophyll molecules $(BChl)_2$. This made determination of the orientation of the chlorophyll molecules from the triplet orientation difficult. However, in the simplest case of the dimer being formed by two coplanar BChl molecules, the

TABLE I

SUMMARY OF RESULTS

Component	g	Orientation maximum	Notes
Q_A^- Fe	1 82 1.66 ?	90° 90°	decreased by pH increase
Q_A^- Fe	1.90 1.66 ?	90° 90°	favoured by pH increase
Split Ph^-	2.00	0° (splitting max)	
S_2 multiline	2.00	splitting maximum in wings at 45°	reflect some anisotropic character (see also Ref. 19)
g 4 donor	4.1	a field shift in light-induced signal. 0° spectrum shifted to low field	reflects its anisotropic character
Cyt b-559 (low-spin)	$g_z \approx 2.97$ $g_y = 2.22$	0° 90°	haem plane oriented perpendicular to the plane of the membrane (see also Refs. 4, 14, 15, 50)
Cyt b-559 (high-spin)	≈ 6.0 (≈ 5.7)	40–50° (90°)	a denatured form induced by drying; haem oriented differently from native form, haem plane 45° to membrane
Signal II	≈ 2.0037 ≈ 2.0062	0° 90°	apparent splitting and g-shifts. Resolves into two four-line spectra which are symmetrical about the g values given. Aromatic ring perpendicular to membrane (see Ref 18)
P-680 triplet	≈ 2.002 Z peaks Y peaks X peaks	 90° 0° 0°	the chlorophyll ring is oriented parallel to the membrane. The direction perpendicular to the ring plane is perpendicular to the membrane (see also Ref 70)

magnetic axes of the triplet state should be analogous to their location in monomeric BChl ring plane. Thus, the special pair was suggested to be oriented in the membrane in such a way that the ($BChl_2$) was nearly perpendicular to the membrane, with the direction perpendicular to the BChl ring plane(s) tilted 10–20° away from the membrane plane [6,7].

The ^{3}P-680 signal can be photoinduced in oriented multilayers of PS II membranes that have been incubated and frozen in a dithionite solution. The triplets signals were small, and averaging and subtraction was necessary. The small size of the signal is partly due to the poor level of reduction obtained in multilayers (see above) but also due to the low quantum yield of triplet observed in relatively intact preparations [63]. Even so, a marked orientation dependence of signal amplitude is observed (Fig. 8). The large outer *Z* peaks were maximal when the membranes were oriented perpendicular to the magnetic field, while both the *X* and *Y* peaks were maximal when the membranes were oriented parallel to the magnetic field. Although the signal-to-noise ratio does not allow very accurate data to be obtained, the results allow the approximate orientation of ^{3}P-680 to be obtained. The structure of P-680 is not known, but both monomeric [60,66] and dimeric [67–69] structures have been proposed. Recent work has suggested that the singlet state may be dimeric, but that the triplet is localised on one of the molecules of the chlorophyll dimer [69]. Assuming a monomeric structure for ^{3}P-680 [60,69], the orientation data in Fig. 8 indicate that the chlorophyll ring plane is in the plane of the membrane.

There is only one other report on the orientation of P-680 [70] measured in magnetically oriented chloroplasts by the absorbance change at 825 nm due to flash-induced P-680$^+$ formation and decay at low temperature. It was concluded that the Q_Y transition (i.e., the plane of the chlorophyll macrocycle) was tilted less than 20° from the membrane plane (i.e., approximately parallel [70]). This agrees with the more extensive EPR data reported here.

The results for ^{3}P-680 are markedly different from those obtained for the analogous signal in purple bacteria.

Summary of results

A summary of the results obtained from this study is presented in Table I.

Conclusion

Nearly all the EPR components observable by EPR in PS II membranes exhibit oriented EPR signals, indicating that they are arranged in the membrane with a fixed geometry.

The orientation data provide several forms of information. Firstly, the geometry of some of the components can be determined. The EPR data shows that the haem plane of cytochrome *b*-559 in its native form is perpendicular to the membrane in agreement with previous work done on chloroplasts [4,14,15]. It is also suggested that the semiquinone ring of the component giving rise to Signal II_s is oriented perpendicular to the membrane (see also Ref. 59). In contrast, the chlorophyll macrocycle of ^{3}P-680 is in the plane of the membrane. These results are significant because they show that the rapid electron transfer reactions between P-680 and Ph and between cytochrome *b*-559 and P-680$^+$ occur between planar redox groups which are perpendicular to each other. This is also the case for the analogous reaction in purple photosynthetic bacterial reaction centres [11]. Secondly, oriented signals have increased amplitudes and thus previously unobserved or poorly resolved features can be observed. Here, large high-field minima in the Q_A^-Fe spectrum which were previously difficult to resolve [21,27,29] are reported. Thirdly, the observation of orientation phenomena in signals where the electronic (or chemical) structure is unknown or only partially understood (i.e., Q_A^-Fe, split Ph^-, S_2 multiline, $g = 4$ signal) provides information that might be useful in future attempts to explain their origins.

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