Biochimica et Biophysica Acta, 1019 (1990) 128–132
Elsevier

BBABIO 43258

Orientation of P700, the primary electron donor of Photosystem I

A.W. Rutherford and P. Sétif

Service de Biophysique, Département de Biologie, Centre d'Etudes Nucléaires de Saclay, Gif-sur-Yvette (France)

(Received 3 April 1990)

Key words: Chlorophyll; Chloroplast orientation; Reaction center; Photosynthesis

Ordered multilayers of Photosystem I particles were obtained by partial drying on mylar sheets. This material was used to study the orientation of P700, the primary electron donor, by monitoring its light-induced triplet state at 4 K by EPR. The orientation dependence of the P700 triplet state was interpreted as indicating that P700 is oriented perpendicular to the mylar sheet. From a comparison of the orientation dependence of the iron-sulphur centres with data reported earlier in ordered chloroplast membranes, it is shown that the Photosystem I particles are ordered on mylar with an orientation comparable to that in the native membrane. Thus it is deduced that P700 is oriented perpendicular to the thylakoid membrane.

Introduction

P700 is the component that is oxidized first when Photosystem I (PS I) is excited by light (reviewed in Refs. 1–3). It consists of chlorophyll *a* with a long-wavelength absorption maximum at 700 nm. There is some controversy over whether P700 is a monomer or dimer of chlorophyll *a* molecules but the current balance of opinion is tipping towards a dimer (reviewed in Refs. 1–3, see also Ref. 4 for recent experimental evidence). The orientation of P700 has been extensively studied using optical techniques (reviewed in Ref. 5). The *y*-axis of P700 has been shown to be in the plane of the chloroplast membrane [6], but since this axis is in the plane of the chlorophyll macrocycle this result does not provide information on the orientation of the chlorophyll plane relative to the plane of the membrane.

Under certain conditions in which forward electron transfer is blocked, excitation of PS I results in the formation of a radical pair, $P700^+A_0^-$, (in which A_0 is the primary electron acceptor). This radical pair decays by populating an usually polarised triplet state of P700 (^{3}P700) [7,8]. Since ^{3}P700 is long-lived (approx. 1 ms at 4 K [9]) relative to the radical pair from which it originates, illumination by continuous light at low temperature allows the triplet signal to be detected by standard EPR techniques [8].

The ^{3}P700 EPR signal is highly anisotropic and thus it can be used to obtain information on the orientation of the chlorophyll macrocycle. In this work PS I particles in ordered multilayers have been used in a study of ^{3}P700 and the orientation of P700 has been determined.

Material and Methods

Two different kinds of PS I particle were prepared from spinach chloroplasts. The first preparation was isolated by digestion with the detergent digitonin [10]. These particles retain their iron-sulphur centres, which act as electron acceptors. The second preparation, isolated using SDS [11], lacked the iron-sulphur centres. In both cases the particles were suspended in distilled water, spread onto sheets of mylar and partially dried in an 80% humidity atmosphere in the dark at 4°C. The drying time varied from 2 to 6 days. Several of the mylar sheets were put into EPR tubes and spectra were recorded at different orientations of the mylar sheets relative to the EPR magnetic field [12].

To obtain a reducing environment the sheets were submerged in a 70% glycerol solution containing sodium dithionite (20%, w/v) in 200 mM glycine (pH 10), incubated in darkness for 5 min and frozen in darkness. Samples were illuminated in the EPR cryostat and in some cases, in a solid CO_2/ethanol bath (200 K) or in a water bath (20°C) using a 500 W projector. Infrared

Abbreviations: A_0, a chlorophyll molecule acting as the primary electron acceptor in PS I; A_1, a secondary electron acceptor; F_A, F_B, F_X, a series of tertiary electron acceptors which are iron-sulphur centres; P700, the primary electron donor of PS I; PS I, Photosystem I; SDS, sodium dodecyl sulphate.

Correspondence: A.W. Rutherford, Service de Biophysique, Département de Biologie, Centre d'Etudes Nucléaires de Saclay, 91191 Gif-sur-Yvette Cedex, France.

radiation was diminished by using a 1 cm water filter and 3 calflex (Balzers) heat filters. EPR spectra were obtained using either a Bruker ER-200 or a Bruker ESP 300 spectrometer fitted with an Oxford Instruments liquid helium cryostat and temperature control system.

Results

Since membrane-free PS I particles were used in this study, it was necessary to establish that they order upon drying with the same orientation as they had in the native membrane. The orientation of the EPR signals arising from the iron-sulphur centres, F_A, F_B and F_X have been studied in some detail in a number of different kinds of biological material ordered by partial drying and by freezing in a magnetic field [13–16]. In the PS I particles isolated using digitonin, the orientation of F_A, F_B and F_X were monitored in the presence and absence of dithionite and before and after illumination at 5 K, at 200 K and at room temperature. The data obtained were essentially the same as reported earlier in chloroplasts. Striking orientation-dependent changes in the intensities of the EPR signals were seen. Because of the similarities to earlier results these effects will not be described in detail here. Fig. 1 shows a relevant example of the orientation effects seen in digitonin particles. The sample was reduced with dithionite and illuminated at 200 K to photoaccumulate the reduced form of F_X. The $g = 1.76$ signal, which is attributed to the g_x component of reduced F_X, is maximum when the mylar sheet is perpendicular to the magnetic field. From the similarities between these data and the orientation dependence for the iron-sulphur centres reported earlier [13–16], it is clear that the PS I particles are oriented relative to the mylar sheet in the same way as they were in the native membrane.

The EPR signal arising from the triplet state of P700 could be observed when the oriented PS I sample was illuminated at 4 K (Fig. 2a). The sample was preilluminated at room temperature and then dark-adapted prior to freezing. This treatment was performed to optimise the yield of the triplet by ensuring that the menaquinone acceptor (A1) is double reduced [17]. The *Z* peaks showed a clear maximum when the mylar sheets were oriented parallel to the magnetic field. The *X* and *Y* peaks, however, were both weaker in amplitude and they overlapped with signals of the reduced iron-sulphur centres. This made determination of their orientation dependencies difficult. Heat-induced changes in the signals of the iron-sulphur centres perturbed the light-minus-dark spectra. It was thus not possible to obtain good data for the orientation of the *Y* peaks. A plot of the amplitude of the *X* peaks gave a poorly defined maximum at around 45°. A signal of unknown origin, seen as a pair of broad troughs around 320 mT and 355 mT, respectively, interfere with the

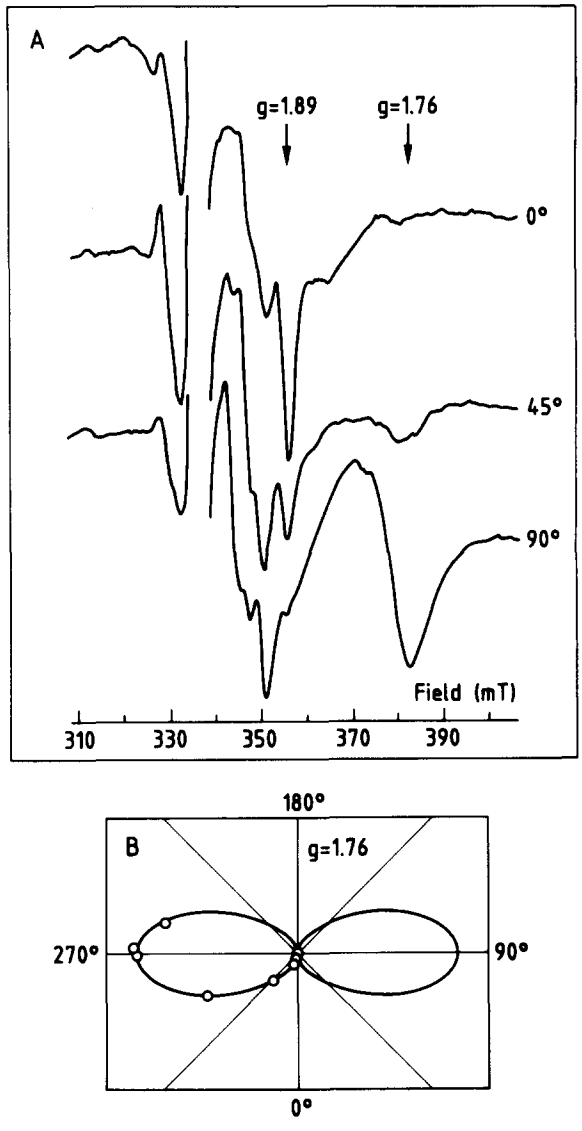

Fig. 1. (A) Orientation dependence of the iron-sulphur centre EPR signals in partially dried PS I particles isolated using digitonin. Several mylar sheets were submerged in a 20% (w/v) sodium dithionite (200 mM glycine/NaOH buffer (pH 10)) and incubated for 5 min in darkness before freezing. Illumination at 200 K for 1 min was given prior to recording the spectra. The angle of the mylar sheet relative to the magnetic field is marked on the figure. EPR instrument settings were as follows: modulation amplitude, 20 G; microwave power, 8 dB; temperature, 8 K. (B) A polar plot of the orientation dependence of the EPR signal at $g = 1.76$.

triplet spectra at orientations close to 90°. The presence of this signal also contributes to the difficulties encountered in determining the orientation maxima for the *X* and *Y* peaks.

In an attempt to improve the quality of the data, PS I particles were used which were prepared using SDS. This kind of preparation lacks the iron-sulphur centres and ^{3}P700 can be detected as well resolved *Z*, *X* and *Y* peaks, even in samples in the absence of dithionite [8,9].

Fig. 3 shows the ^{3}P700 signal in this kind of preparation when dried on mylar. Although not as well ordered as in digitonin particles, the *Z* peaks still showed an amplitude maximum when the sheets were oriented parallel to the magnetic field. This confirms the observation made in digitonin particles. The *Y* peaks were easily detected and their amplitudes were close to maximum when the mylar was oriented at 60–70° to the magnetic field. The data for the *X* peaks were rather poorly resolved but are reconcilable with a maximum at 20–30°, as predicted from the rule that the squared cosines of the three axis angles relative to the membrane normal must add up to 1 [14]. These results also seem reconcilable with the poorer data obtained for the *X* and *Y* peaks in digitonin PS I particles (Fig. 2).

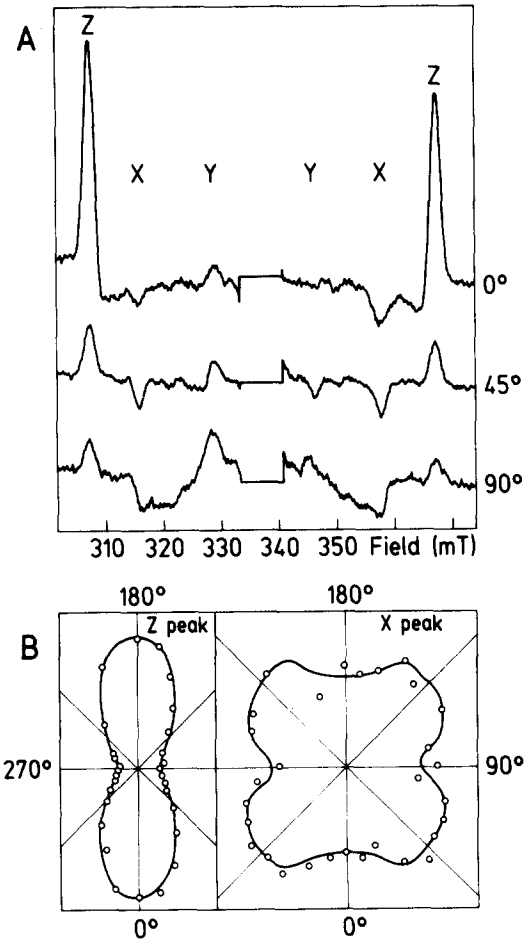

Fig. 2. (A) Orientation dependence of the light-induced P700 triplet EPR signal in reduced (digitonin) PS I particles. The sample was treated as described in the legend to Fig. 1 except that no illumination at 200 K was given and, instead, 4 min of illumination were given at room temperature, followed by 1 min of dark adaptation, prior to freezing in darkness. The figure shows light-minus-dark spectra recorded at 4.2 K with the following instument settings: modulation amplitude, 18 G; microwave power, 40 dB; eight accumulations. (B) Polar plot of the orientation dependence of the *Z* and *X* peaks of the triplet signal in PS I (digitonin) particles. The amplitude of the *X* peak has been multiplied by approx. 5 times relative to that of the *Z* peak.

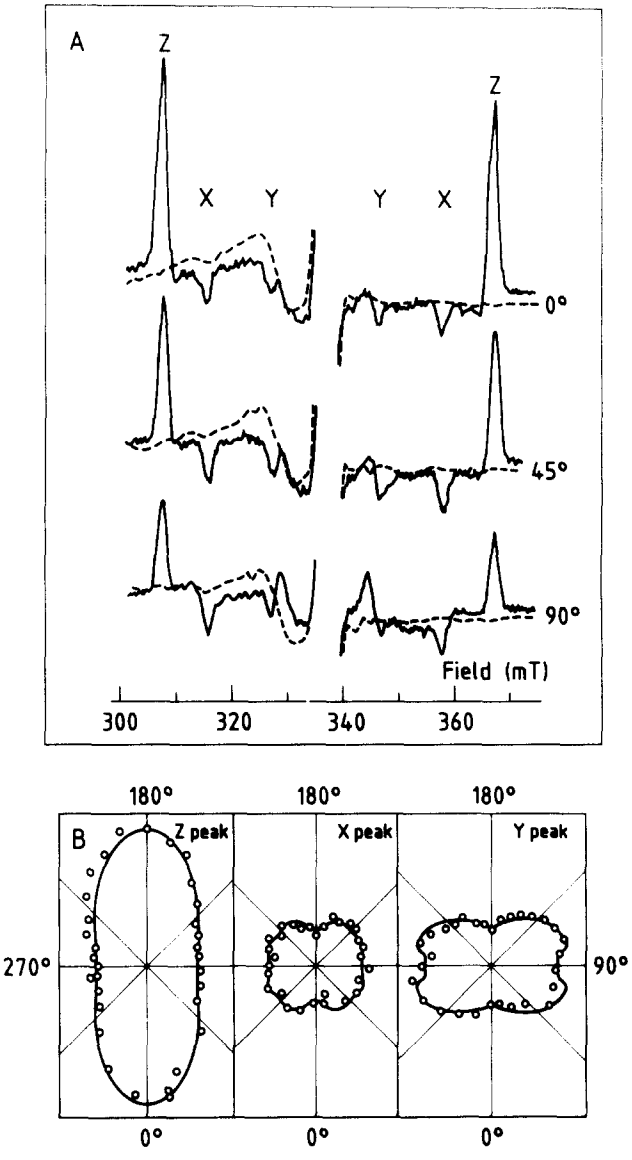

Fig. 3. (A) orientation dependence of the P700 triplet EPR signal in SDS particles. Samples were treated with sodium dithionite as described in the legend to Fig. 1. The dark-adapted sample was cooled to 4.2 K in darkness and spectra were recorded under illumination (solid lines) and in the dark, after illumination (broken lines). EPR instrument settings were as follows: modulation amplitude, 10 G; microwave power, 40 dB (20 μW); temperature, 4.2 K. (B) Polar plots of the three triplet peaks. In the plots, the amplitudes of the *X* and *Y* peaks have been multiplied by 2 relative to the amplitude of the *Z* peak.

Discussion

It is clear that in both PS I preparations the Z peak of ^{3}P700 is maximum when the mylar is oriented perpendicular to the magnetic field. From the measurements of the iron-sulphur centres (Fig. 1), it is also clear that the PS I particles are oriented as they were in the

native membrane. From magneto-photoselection experiments, it is known that the Z axis of the triplet in chlorophyll is perpendicular to the plane of the tetrapyrrole ring [18]. Thus, the straightforward interpretation of the data is that the chlorophyll which consitutes ^{3}P700 is oriented perpendicular to the membrane.

Since the question of whether P700 is a monomer or a dimer is unresolved, the influence of the structure of P700 on the interpretation of the orientation data must be considered. If P700 is a monomer, the interpretation given above is valid. If, however, as seems more likely, P700 is a dimer, the situation is somewhat less clear.

If the triplet is localized on one half of the dimer, the orientation data are again straightforward to interpret: the chlorophyll, on which the triplet is localized, is perpendicular to the membrane. In this case, no information on the other chlorophyll of the dimer is obtained. If, on the other hand, the triplet is shared over both chlorophylls of the dimer, the orientation of the triplet axes would be an average of the axes of the constituent chlorophylls [19]. Thus the data obtained could reflect a non-obvious orientation of the two chlorophylls.

When the equivalent measurements were made in purple bacteria, the same problem had to be considered [20,21]. It was suggested that the most likely structure for the special pair was a pair of bacteriochlorophyll molecules which were parallel to each other. The triplet orientation data were thus taken as an indication that both bacteriochlorophyll molecules were almost perpendicular to the membrane [20,21]. X-ray crystallography confirmed this interpretation [22]. Similar considerations and interpretations may be valid for P700 since recent evidence indicates closer structural similarities between PS I and purple bacteria than was previously thought [23,24].

A recent study of the reaction centre of green sulphur bacteria indicated that it can be considered as an evolutionary 'missing link' between the purple bacterial reaction centre and that of PS I [24]. It was thus suggested that new insights on the structure of the PS I reaction cente could be had by assuming a structural analogy with the well-characterised reaction centre of purple bacteria [24]. In addition, from a comparison of the amino acid sequences of purple bacterial reaction centre proteins with those of PS I, conserved histidines were pointed out as possible ligands to P700 [23]. These results implied that P700 was a special pair of chlorophyll molecules structurally analogous to the primary electron donor in purple bacterial reaction centres.

If P700 is a pair of chlorophyll molecules, an explanation should be found for the monomeric zero field splitting parameters exhibited by the P700 triplet [7,8]. A simple explanation could be that the triplet is localized on one half of the chlorophyll special pair. Alternatively, in purple bacteria, it was concluded that the decrease in the zero field splitting parameters of the triplet state was due largely to its charge transfer character rather than its delocalization [19]. Thus, even if the P700 triplet were delocalized over two chlorophyll molecules, it would show monomeric zero field parameters if it lacked charge transfer character. Such differences in the nature of the triplet in a chlorophyll special pair, compared to those of the triplet in a bacteriochlorophyll special pair, may originate from the properties of chlorophyll versus those of bacteriochlorophyll. It was pointed out earlier that the substitution of the acetyl group on ring 1 in bacteriochlorophyll by a vinyl group in chlorophyll could have an influence on the localization of the triplet in a chlorophyll special pair [25]. This structural difference could also determine the magnitude of any charge transfer character.

Acknowledgements

We would like to thank J. Breton and particularly W. Nitschke for helpful discussion. This work was supported in part by the C.N.R.S. (U.R.A.1290).

References

1 Rutherford, A.W. and Heathcote, P. (1985) Photosynth. Res. 6, 295–316.
2 Sétif, P. and Mathis, P. (1986) in Encyclopedia of Plant Physiology, New Series (Staehlin, A. and Arntzen, C.J., eds.), Vol. 19, p. 476–486, Springer, Berlin.
3 Golbeck, J.H. (1988) Biochim. Biophys. Acta 895, 167–204.
4 Moënne-Loccoz, P., Robert, B., Ikegami, I. and Lutz, M. (1990) Biochemisty, 29, 4740–4746.
5 Breton, J. and Vermeglio, A. (1982) in Photosynthesis: Energy Conversion by Plants and Bacteria (Govindjee, ed.) Vol. 1, pp. 153–194, Academic Press, New York.
6 Breton J. (1977) Biochim. Biophys. Acta 459, 66–75.
7 Frank, H.A., McLean, M.B. and Sauer, K. (1979) Proc. Natl. Acad. Sci. USA 76, 5124–5128.
8 Rutherford, A.W and Mullet, J.E. (1981) Biochim. Biophys. Acta 635, 225–235.
9 Sétif, P., Quaegebeur, J.P. and Mathis, P. (1982) Biochim. Biophys. Acta 681, 345–353.
10 Boardman, N.K. (1971) Methods Enzymol. 23, 268–276.
11 Sétif, P., Acker, S., Lagoutte, B. and Duranton, J. (1980) Photosynth. Res. 1, 17–27.
12 Blasie, J.K., Erecinska, M., Samuels, S. and Leigh, J.S. (1978) Biochim. Biophys. Acta 501, 33–52.
13 Dismukes, G.C. and Sauer, K. (1978) Biochim. Biophys. Acta 504, 431–445.
14 Prince, R.C., Crowder, M.S. and Bearden, A. (1980) Biochim. Biophys. Acta 592, 323–337.
15 Hootkins, R. and Bearden, A. (1983) Biochim. Biophys. Acta 723, 16–29.
16 Aasa, R., Bergström, J. and Vänngard, T. (1981) Biochim. Biophys. Acta 637, 118–123.
17 Sétif, P. and Bottin, H. (1989) Biochemistry 28, 2689–2697.
18 Thurnauer, M.C. and Norris, J.R. (1977) Chem. Phys. Lett. 47, 100–105.
19 Norris, J.R., Budil, D.E., Gast, P., Chang, C.-H., El-Kabbani, O. and Schiffer, M. (1989) Proc. Natl. Acad. Sci. USA 86, 4335–4339.

20 Hales, B.J. and Gupta, A.D. (1979) Biochim. Biophys. Acta 548, 276–286.
21 Tiede, D.M. and Dutton, P.L. (1981) Biochim. Biophys. Acta 637, 278–290.
22 Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1984) J. Mol. Biol. 180, 385–398.
23 Robert, B. and Moenne-Loccoz, P. (1990) in Current Research in Photosynthesis (Baltscheffsky, M., ed.), Vol. 1, pp. 65–68, Kluwer, Dordrecht.
24 Nitschke, W., Feiler, U. and Rutherford, A.W. (1990) Biochemistry 29, 3834–3842.
25 Rutherford, A.W. (1986) Biochem. Soc. Trans. 14, 15–17.