Biochimica et Biophysica Acta, 1058 (1991) 379–385

ADONIS 000527289100172J

BBABIO 43440

A chlorophyll tilted 30° relative to the membrane in the Photosystem II reaction centre

F.J.E. van Mieghem [1,2], K. Satoh [3] and A.W. Rutherford [1]

[1] *Section de Bioénergétique, Département de Biologie Cellulaire et Moléculaire, CE Saclay, Gif-sur-Yvette (France),* [2] *Department of Molecular Physics, Agricultural University, Wageningen (The Netherlands) and* [3] *Department of Biology, Faculty of Science, Okayama University, Okayama (Japan)*

(Received 24 January 1991)

Key words: Triplet state; EPR; Photosynthesis; Primary electron donor

The orientation properties of the reaction centre triplet in Photosystem II (PS II) were determined. A high triplet yield was generated in PS II membranes by double reduction of the primary quinone electron acceptor Q_A. It is deduced that the triplet state is localised on a chlorophyll, the tetrapyrrolic plane of which is tilted at 30° to the membrane. A similar orientation was found in D1/D2/cyt *b*-559 particles demonstrating that the triplet is confined to the reaction centre. Optical work in the literature has been interpreted as indicating that the triplet is localised on a monomeric chlorophyll and that, in the singlet state, P680 consists of this molecule weakly coupled to a second chlorophyll. The weakness of the coupling, compared to the coupling in the special pair of purple bacteria, allows P680 to be considered as a monomer. Taking the optical data into account, we propose that P680 is a chlorophyll molecule oriented at 30° to the membrane. This result is discussed in terms of the structural analogy between PS II and the reaction centre of purple bacteria. A model is favored in which P680 is a chlorophyll, structurally analogous to one of the monomeric bacteriochlorophylls of the bacterial reaction centre. In addition, the orientation data indicate that this chlorophyll is rotated by 45° in its ring plane compared to the monomeric bacteriochlorophylls in the reaction centre of *Rhodopseudomonas viridis*.

Introduction

The reaction centre of purple bacteria consists of three subunits, H, L and M. The primary photochemical reactions take place in the L and M subunits that form a heterodimer. They bind several pigments: four bacteriochlorophylls, two bacteriopheophytins, two quinones and additionally one iron atom. Most species also bind a carotenoid molecule. The crystal structure of the reaction centre from *Rhodopseudomonas viridis* and *Rhodobacter sphaeroides* have been resolved and an almost symmetrical distribution of these components over the L M heterodimer was shown (reviewed in Refs. 1, 2).

After excitation of the reaction centre with light, a radical pair is formed within a few picoseconds: the positive charge is distributed over a pair of bacteriochlorophylls known as the special pair and the negative charge is located on a bacteriopheophytin on the L side of the reaction centre. In reaction centres where the quinone acceptor Q_A is present, electron transfer from the bacteriopheophytin anion to Q_A occurs in 200 ps. When this electron transfer step is blocked, spin dephasing and then charge recombination of the radical pair can occur, transiently forming a triplet state on the special pair of bacteriochlorophylls (reviewed in Ref. 3).

The special pair is located on the symmetry axis of the reaction centre; the two bacteriochlorophylls have their ring planes almost parallel to this symmetry axis, which means that they are perpendicular to the membrane. There are two monomeric bacteriochlorophylls that are located between the special pair and the

Abbreviations: Q_A, the first quinone electron acceptor; PS II, Photosystem II; P680, the primary electron donor in PS II; PS I, Photosystem I; Chl, chlorophyll; Bchl, bacteriochlorophyll.

Correspondence: A.W. Rutherford, Section de Bioénergétique, Département de Biologie Cellulaire et Moléculaire, CE Saclay, 91191 Gif-sur-Yvette Cedex, France.

bacteriopheophytins, on each side of the symmetry axis. From its position, it seems likely that the monomer on the L side is involved in the electron transfer [1,2]; whether or not it acts as a true electron acceptor is still under debate [4,5].

On the basis of spectroscopic data, there are thought to be many similarities in structure and function between purple bacteria and PS II (reviewed in Refs. 6–8). Based on the similarities of amino acid sequences between D_1 and D_2 in PS II and the L and M subunits in bacteria, a specific folding model for the PS II reaction centre was proposed [9–11]. The proposal that D_1 and D_2 make up the reaction centre was supported by some biochemical evidence (e.g. [12]), but was established by the isolation of a PS II reaction centre core made up of only D_1, D_2, cytochrome *b*-559 [13]. The validity of the folding model was established by site-directed mutagenesis experiments [14,15].

From amino acid sequence comparisons it is clear that the histidines that are the axial ligands to the bacteriochlorophylls of the special pair in purple bacteria, are conserved in PS II [9,10]. It thus seems probable that there is at least a structural equivalent of the bacterial special pair in PS II. It is not clear, however, whether structural equivalents of the monomeric bacteriochlorophylls exist in PS II. In purple bacteria these have histidine residues as axial ligands, each of which is located at the end of a helix that is parallel to the membrane (the so called CD helix) [1], but these histidines are absent in PS II [9,10]. However, in *Rb. capsulatus*, these histidines have been replaced by several other amino acids using site-directed mutagenesis. A preliminary analysis of these mutants indicated that axial histidines are not required for binding of the monomeric bacteriochlorophylls to the reaction centre (reviewed in Ref. 16). Therefore, the fact that histidines in the predicted CD helix of PS II are absent, does not argue against the presence in PS II of monomeric chlorophylls, which are structurally analogous to those in the bacterial reaction centre. Moreover, the existence of a monomeric chlorophyll on the active side of the reaction centre might be predicted on theoretical grounds, based on the rate of electron transfer [17].

From orientation studies of the PS II triplet EPR signal, it was concluded that the triplet at liquid helium temperatures is localised on a chlorophyll molecule, the ring plane of which is oriented approximately parallel to the membrane [18,19]. This is one of the most striking spectroscopic differences between PS II and purple bacterial reaction centres, since, in the latter, the triplet resides on the pair of bacteriochlorophylls which are perpendicular to the membrane [20,21]. Also, in all other types of photosynthetic reaction centres, PS I, green sulphur bacteria and heliobacteria, the triplet is localised on (bacterio)chlorophylls which are perpendicular to the membrane plane, as in purple bacteria [22–24]. It was possible to interpret the orientation data on the triplet of PS II in the context of a strict structural analogy between PS II and purple bacteria by assuming that, in PS II, the triplet is localised on a structural equivalent of one of the monomeric bacteriochlorophylls. In this model the triplet was envisaged either (1) as being transferred from P680 to this chlorophyll monomer [6] or (2) the monomer could be P680 itself [25].

The original orientation experiments were done with preparations in which Q_A was prereduced to block further electron transfer after charge separation [18,19]. It is now known, however, that the presence of Q_A^- almost completely suppresses triplet formation in PS II [26]. By doubly reducing Q_A, the triplet yield can be increased drastically. This effect was attributed to a low yield of the primary radical pair when Q_A^- is present, due to a repulsive coulombic interaction between this radical pair and Q_A^- [26] (see also Refs. 27, 28). When Q_A is doubly reduced, it presumably loses its negative charge by protonation, resulting in an increase of the triplet yield. Similarly, complete removal of Q_A results in a high triplet yield as seen in the D_1/D_2/cyt *b*-559 preparation [29]. Since the conditions under which the triplet can be generated are better understood, we are now able to make oriented PS II samples having triplet signals with a much greater intensity than in earlier work. Two types of preparations with increased triplet yield were used: PS II membranes with Q_A doubly reduced [26] and a reaction centre preparation with Q_A absent [13]. Thus, we were able to obtain much more precise orientation data on the reaction centre triplet.

Materials and Methods

PS II-enriched thylakoid membrane fragments were prepared from spinach as described earlier [30] using the modifications in Ref. 31. In order to doubly reduce Q_A, the membranes were maintained in the dark for several hours at a redox potential of approx. −400 mV, under argon, in the presence of approx. 2 mM sodium dithionite [26]. In addition, 5 mM Mops (pH 7.0), 1 mM EDTA and 50 μM benzyl viologen [26] were present. The samples were then spread under argon onto mylar sheets and dried in an 80% humidity, argon atmosphere in the dark at 4°C, for approx. 24 h [32]. D_1/D_2/cyt *b*-559 particles [13] were resuspended in water, directly spread onto mylar sheets and dried in the same way as the membranes, except that a pyrogallol oxygen trap was present in the drying vessel.

Several of the mylar sheets were put into EPR tubes and a 70% glycerol solution containing approx. 100 mM sodium dithionite and 200 mM glycine (pH 10) was added. In some experiments 150 mM Mops (pH

7.0) was used instead of 200 mM glycine. After 5 min dark incubation, the samples were frozen and spectra were recorded at different orientations of the mylar sheets relative to the EPR magnetic field [32]. For the D_1/D_2/cyt *b*-559 particles data were recorded first in the absence of dithionite. Under these conditions, identical data for triplet orientation were recorded but in addition, data on oxidised cytochrome *b*-559 were also obtained.

EPR spectra were recorded using a Bruker 200 X-band spectrometer fitted with an Oxford Instruments cryostat and temperature control system. In order to record spectra during illumination an 800 W tungsten projector was used. Illumination was performed through the cavity window with the light beam perpendicular to the magnetic field. Infrared radiation was diminished by using a 2 cm water filter and three calflex (Balzers) heat filters.

Results

Oriented PS II membranes with Q_A doubly reduced

It is possible to doubly reduce Q_A in PS II membranes by incubating them under strongly reducing conditions (see Materials and Methods for details). The samples, pretreated in this way, were oriented on mylar strips as described in Materials and Methods. The triplet state can be detected under illumination at liquid helium temperature with EPR [33]. A very intense triplet EPR signal was observed as in Ref. 26. The triplet spectra (Fig. 1a) showed an AEEAAE polarisation pattern and *D* and *E* values of 0.0286

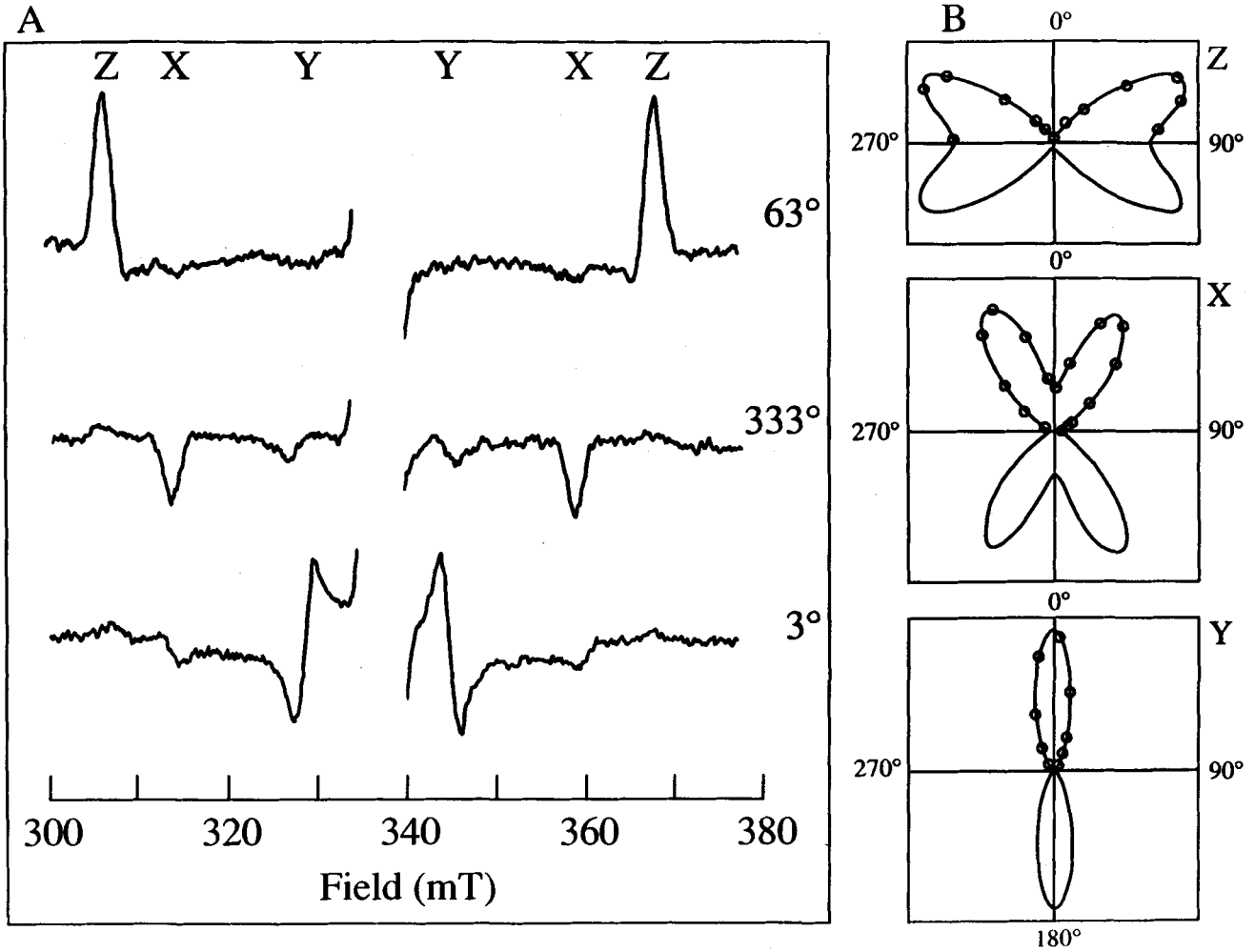

Fig. 1. (A) Orientation dependence of the light-induced PS II reaction centre triplet EPR signal in PS II membranes with Q_A doubly reduced; the sample was reduced with sodium dithionite (see Materials and Methods for details on sample preparation). The figure shows spectra recorded close to the orientation maximum for each of the triplet peaks; the angle between the magnetic field and the plane of the mylar sheets is indicated for each spectrum. The spectra were recorded during illumination with the following instrument settings: temperature, 4.4 K; modulation amplitude, 25 G; gain, $1 \cdot 10^5$; microwave power, 35 dB (63 μW); microwave frequency, 9.434 GHz. (B) Normalised polar plots of the orientation dependence of the Z, X and Y triplet peaks, obtained under the conditions outlined under A.

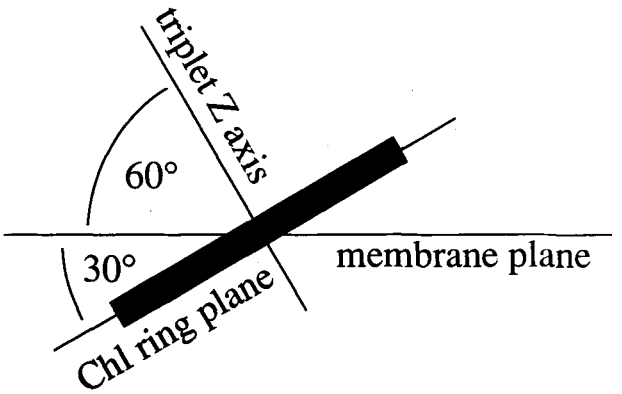

Fig. 2. A schematic drawing (side view) showing the orientation of the chlorophyll on which the triplet state is localised in PS II. The triplet Z axis makes an angle of 60° with the membrane plane, as reported in this work, and is perpendicular to the ring plane of the chlorophyll [36]. The ring plane, therefore, is oriented at 30° with respect to the membrane.

cm^{-1} and 0.0044 cm^{-1} respectively. These are characteristics of the reaction centre triplet of PS II [33,34]. When the angle between the mylar strips and the magnetic field was varied, the amplitudes of the triplet peaks showed a marked orientation dependence. This is shown as polar plots in Fig. 1b.

The triplet Z, X and Y peaks showed maxima at angles of 63°, 30° and 0°, respectively between the plane of the mylar strips and the magnetic field. The orientation dependence of the Z peaks was slightly less well resolved and this may be due to a specific increase in light penetration of the sample when the beam was parallel to the mylar strips (i.e., when the strips were 90° to the magnetic field). This would result in a distortion of the orientation dependence due to an increased Z peak signal amplitude at this orientation. The other peaks are essentially unaffected since they have almost zero intensity at this orientation.

It is also possible to calculate the orientation maximum of the Z peaks from those of the X and Y peaks, using the relationship [35]:

$$\sum_i \cos^2(90° - \alpha_i) = 1 \qquad (1)$$

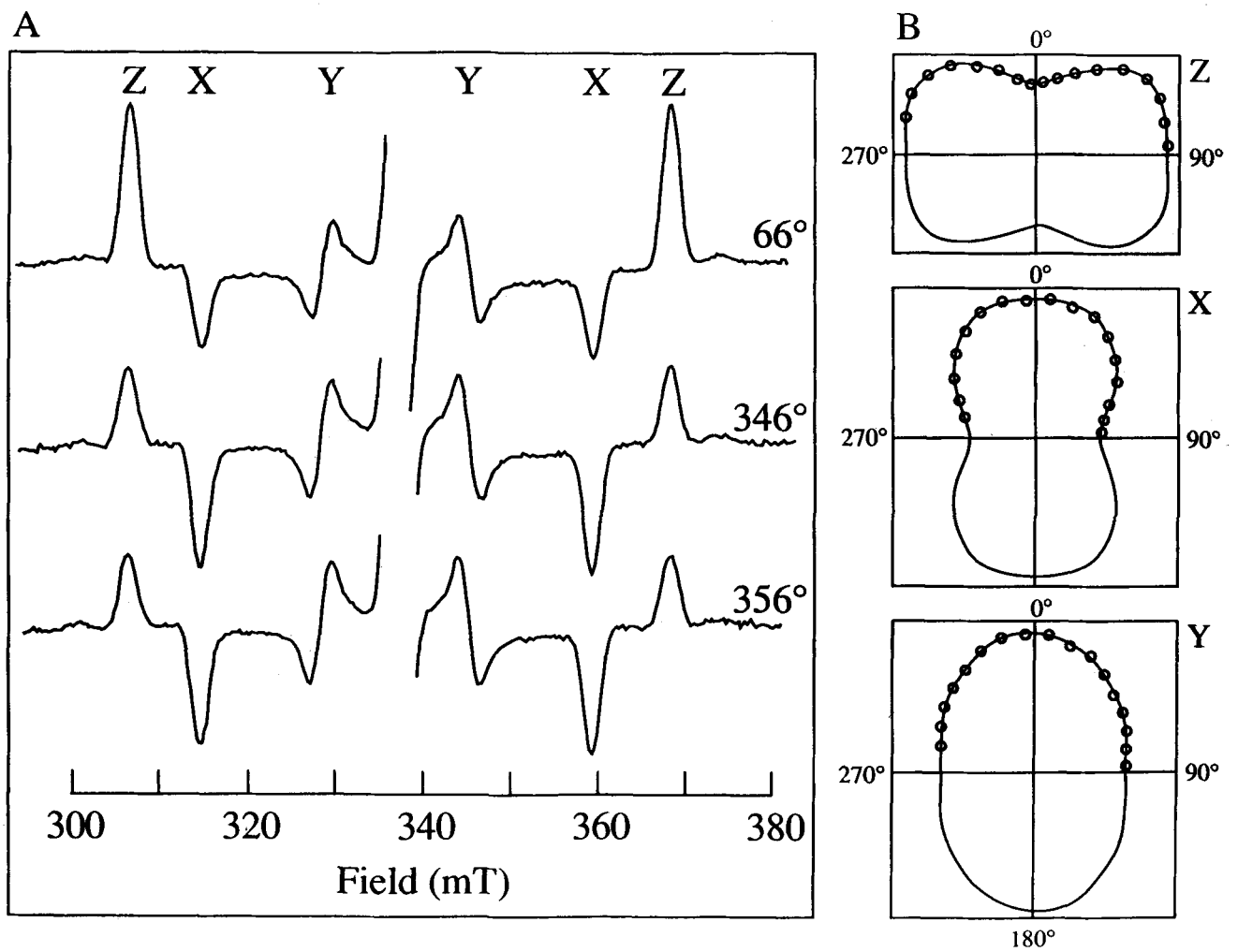

Fig. 3. (A) Orientation dependence of the light-induced PS II reaction centre triplet EPR signal in D_1/D_2/cyt *b*-559 particles. Sample conditions and instrument settings were as described in the legend to Fig. 1, except that the gain was $5 \cdot 10^4$. (B) Normalised polar plots of the three triplet peaks from D_1/D_2/cyt *b*-559 particles.

(where α_i is the orientation maximum for respectively the Z, X and Y peak). This yields an angle of 60°, which we take as a more accurate value (±3°, estimated error). We conclude that the angles of the triplet Z, X and Y axes with the plane of the thylakoid membrane are respectively 60°, 30° and 0°.

The triplet axis system of chlorophylls in vitro has previously been determined [36]. It was found that the triplet Z axis is perpendicular to the ring plane for all types of chlorophylls. Furthermore, we assume that the triplet is entirely located on a single chlorophyll, based on the monomeric *D* value [33,34], the triplet minus singlet spectrum [29,34,37,38] and the weakness of the coupling between the pigments in the PS II reaction centre [38–40]. Thus, we deduce that the ring plane of this chlorophyll is tilted at an angle of 30° relative to the plane of the membrane (see Fig. 2).

Oriented D_1/D_2/cyt b-559 particles

Due to the absence of Q_A in these particles, the triplet yield is high without preincubation under reducing conditions [29,41] (see Ref. 26). The samples were oriented on mylar strips in the same way as the PS II membranes (see above) and EPR spectra of the triplet state were taken during illumination at liquid helium temperature. Essentially the same spectral features as in PS II membranes were observed (Fig. 3a). Fig. 3b shows polar plots of the Z, X and Y peaks of the triplet in oriented D_1/D_2/cyt *b*-559 particles. Due to the fact that the particles were extracted from the membrane, the samples showed a less defined orientation dependence compared to PS II membranes. The orientation dependence of EPR signals from the oxidised cytochrome *b*-559 in these samples (not shown) was as in PS II membranes [18], confirming that the particles were oriented with the symmetry axis of the reaction centre perpendicular to the mylar plane. Despite the lower quality of the data, it is still observable that the orientation maximum for the Z peak deviates 20°–30° from 90°. This is consistent with the triplet residing on a chlorophyll molecule that is tilted approx. 30° out of the plane of the mylar, as found for oriented membranes.

Discussion

The data presented here confirm the earlier observations [18,19] that the reaction centre triplet of PS II has a different orientation compared to all other photosynthetic reaction centres. An orientation parallel to the membrane was reported for the ring plane of the chlorophyll on which the triplet was located, but the signal-to-noise in the earlier work was insufficient to allow this interpretation to be very precise. In the present study, the pretreatment of the sample allowed the triplet yield to increase by orders of magnitude. The signal to noise is correspondingly improved and it is now clear that the ring plane of the chlorophyll on which the reaction centre triplet is localised is tilted 30° out of the membrane plane.

The observation that the orientation dependence of the triplet in the isolated PS II reaction centre (D_1/D_2/cyt *b*-559) is the same as that in the unfractionated membranes, is a clear indication that the triplet resides on the same reaction centre chlorophyll molecule in both preparations. Furthermore, it is evident that the components involved in the primary reactions in the D_1/D_2/cyt *b*-559 preparation [13] are relatively intact.

In other types of photosynthetic reaction centres, the triplet state is localised on the primary electron donor. The triplet minus singlet spectrum [29,34,37,38], indicates that this is also the case in PS II. It was argued in Refs. 34, 37 and 38 that P680 comprises two excitonically coupled chlorophylls in the singlet state, but that the triplet was localised on only one of the chlorophylls (for a recent review of spectroscopy on P680 see Ref. 42). However, it is important to note here that all of the estimations in the literature for possible excitonic coupling between PS II reaction centre pigments (e.g., 40 cm^{-1} [38], 142 cm^{-1} [39] or 110 cm^{-1} [40]) are low compared to estimations for the coupling within the bacterial special pair (in the range of 600–1500 cm^{-1} [43,44]). In addition, the absence of a significant Stark effect, in contrast to the situation in purple bacteria [45,46], indicates that there are no contributions of charge transfer-states to the excited state [45]. Compared to the situation in the purple bacterial centre, these observations can be taken as indicating that all the pigments in the PS II reaction centre are essentially monomeric. Thus, P680 can be considered as a monomer, although weakly coupled with one or more nearby chromophores. The close resemblance between the $P680^+$ minus P680 spectrum and the $^3P680$ minus P680 spectrum [38] suggests that the triplet state and the positive charge are both localised on the same chlorophyll. If this is the case, this chlorophyll can be considered to be P680 and from the present work, it is oriented at 30° with respect to the membrane plane.

How then should this result be considered in light of the expected structural analogy between PS II and the purple bacterial reaction centre? If, as seems appropriate, we attempt to apply the analogy as strictly as possible, further useful insights on the nature of P680 can be gained. In the following we discuss two different models for the organisation of the P680 region in PS II, in which a major criterion is the maintenance of the structural analogy.

In the first model, two chlorophylls, which are structural analogues to the bacteriochlorophyll special pair, are present in PS II (as suggested by their conserved

histidines), but one of these is oriented at 30° rather than 90° to the membrane plane. Such an arrangement could result in a weaker coupling between the two chlorophylls, giving rise to essentially monomeric properties. However, different tilts of the chromophores of the PS II reaction centre, compared to their purple bacterial counterparts, inevitably imply differences in the protein folding. This seems unlikely in the region where the bacterial special pair is bound, because there, the homology between D_1 and D_2 and the L and M subunits is rather high.

In an alternative model, which was suggested earlier [25], P680 is a chlorophyll molecule structurally equivalent to one of the bacteriochlorophyll monomers of the bacterial reaction centre; two chlorophylls, which are structural analogues to the bacterial special pair, are present in PS II, but they are not the primary electron donor. This model is attractive since a 30° tilt of P680, apparent from the present work, is exactly that seen for the bacteriochlorophyll monomers [1] (see Table I). It is envisaged that the organisation of the chromophores (their positions and the tilts of their ring planes) and the folding of the protein in PS II are essentially the same as in the purple bacterial reaction centre, although different functional roles for some of the chromophores are imagined.

Table I shows orientation data for the monomeric bacteriochlorophylls, taken from the crystal structure of *Rps. viridis* [1], compared to orientation data for the chlorophyll on which the triplet is localised in PS II. It can be deduced that the triplet X and Y axes are rotated * approx. 45° clockwise or 135° counterclockwise within the ring plane, when compared to the directions of the optical transition moments of the bacterial monomers (for chlorophylls, the X and Y triplet axes correspond to the Q_x and Q_y transitions respectively [36]). The most straightforward interpretation for this is that a structural difference is present, involving a rotation of the chlorophyll ring in PS II compared to purple bacteria, while maintaining the 30° tilt of the ring plane. Such a rotational difference is not in contradiction with a close structural analogy between PS II and the purple bacterial reaction centre since it is not likely to have a large impact on the protein folding. It would only require replacement of amino acids in PS II that have close interactions with the chlorophyll and possibly slight modifications due to the phytyl chain taking a different position in the protein compared to purple bacteria. The proposed in-plane rotation could contribute to the functional differences proposed in this model for this chlorophyll, compared to its bacterial counterpart.

* When observing the reaction centre from the donor side.

TABLE I

Orientation of optical axes (Rps. viridis, monomeric bacteriochlorophylls) and triplet axes (PS II) with respect to the membrane

	X-axis	Y-axis	XY-plane
Rps. viridis, optical axes of the monomeric Bchls [a]			
L side	19°	21°	32°
M side	22°	23°	28°
PS II, triplet axes	30°	0°	30°

[a] From Ref. 1.

In order to explain the singlet minus triplet spectrum [29,34,37,38] in line with this model, we consider excitonic coupling to occur between a chlorophyll monomer (structurally analogous to one of the bacteriochlorophyll monomers of the bacterial reaction centre) and one or both of the chlorophylls which are structurally analogous to the constituents of the bacterial special pair. Couplings of 40 cm^{-1} [38], 142 cm^{-1} [39] or 110 cm^{-1} [40] are indeed in the range of the weak couplings which were calculated previously for the interaction of the bacteriochlorophylls of the special pair with the monomeric bacteriochlorophylls in the purple bacterial reaction centre [43,44]. The estimations of the magnitude of the coupling in the PS II reaction centre would also imply that there is only a small interaction in PS II between the structural equivalents of the bacterial special pair. This could occur even if the relative position and tilts of the ring planes are similar in both reaction centres [39] (see also Ref. 47) and thus would not involve major changes of the protein folding. However, the requirement for the structural analogue of the bacterial special pair to be decoupled in PS II in this model, is an ad hoc hypothesis which weakens the model somewhat.

In conclusion, of the two models discussed we favour the second, in which P680 is a chlorophyll molecule which is a structural analogue of one of the monomeric bacteriochlorophylls, because this more closely maintains in PS II the basic structural motif of the bacterial reaction centre.

Acknowledgements

We would like to thank R.E. Blankenship, J. Breton, P. Mathis, T. Mattioli, W. Nitschke, G.F.W. Searle, W.F.J. Vermaas, M.H. Vos and in particular T.J. Schaafsma for useful discussions. Thanks also to G. Renger for sending us a reprint of unpublished work. F.v.M. is supported by the EEC (SCIENCE N.P. Programme) and A.W.R. is suported by the CNRS (URA 1290).

References

1 Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1985) Nature (London) 318, 618–624.
2 Allen, J.P., Feher, J., Yeates, T.O., Komiya, H. and Rees, D.C. (1987) Proc. Natl. Acad. Sci. USA 84, 5730–5734.
3 Parson, W.W. and Ke, B. (1982) in Photosynthesis: energy conversion by plants and bacteria (Govindjee, ed.), pp. 331–385, Academic Press, New York.
4 Fleming, G.R., Martin, J.-L. and Breton, J. (1988) Nature (London) 333, 190–192.
5 Holzapfel, W., Finkele, U., Kaiser, W., Oesterhelt, D., Scheer, H., Stilz, H.U. and Zinth, W. (1990) Proc. Natl. Acad. Sci. USA 87, 5168–5172.
6 Rutherford, A.W. (1986) Biochem. Soc. Trans. 14, 15–17.
7 Rutherford, A.W. (1987) in Progress in Photosynth. Res. (Biggins, J., ed.), Vol. 1, pp. 277–283 Martinus Nijhoff, Dordrecht.
8 Mathis, P. and Rutherford, A.W. (1987) in New Comprehensive Biochemistry: Photosynthesis (Amesz, J., ed.), pp. 63–96, Elsevier, Amsterdam.
9 Trebst, A. (1986) Z. Naturforsch. 41c, 240–245.
10 Michel, H. and Deisenhofer, J. (1988) Biochemistry 27, 1–7.
11 Barber, J. and Marder, J.B. (1986) in Biotechnology and Genetic Engineering Reviews (Russel, M., ed.), Vol. 4, pp. 355–405, Intercept, Newcastle upon Tyne.
12 Metz, J.G., Bricker, T.M. and Seibert, M. (1985) FEBS Lett. 185, 191–196.
13 Nanba, O. and Satoh, K. (1987) Proc. Natl. Acad. Sci. USA 84, 109–112.
14 Debus, R.J., Barry, B.A., Babcock, G.T. and McIntosh, L. (1988) Proc. Natl. Acad. Sci. USA 86, 427–430.
15 Vermaas, W.F.J., Rutherford, A.W. and Hansson, Ö. (1988) Proc. Natl. Acad. Sci. USA 85, 8477–8481.
16 Coleman, W.J. and Youvan, D.C. (1990) Annu. Rev. Biophys. Biophys. Chem. 19, 333–367.
17 Moser, C.C., Keske, J.M., Warncke, K. and Dutton, P.L. (1991) Biophys. J. 59, 521a.
18 Rutherford, A.W. (1985) Biochim. Biophys. Acta 807, 189–201.
19 Rutherford, A.W. and Acker, S. (1986) Biophys. J. 49, 101–102.
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 Rutherford, A.W. and Sétif, P. (1990) Biochim. Biophys. Acta 1019, 128–132.
23 Nitschke, W., Feiler, U. and Rutherford, A.W. (1990) Biochemistry 29, 3834–3842.
24 Nitschke, W., Sétif, P., Liebl, U., Feiler, U. and Rutherford, A.W. (1990) Biochemistry 29, 11079–11088.
25 Rutherford, A.W. (1988) in Light-Energy Transduction in Photosynthesis: Higher Plant and Bacterial Models (Stevens, S.E. and Bryant, D.A., eds.), pp. 163–177, The American Society of Plant Physiologists, Rockville.
26 Van Mieghem, F.J.E., Nitschke, W., Mathis, P. and Rutherford, A.W. (1989) Biochim. Biophys. Acta 977, 207–214.
27 Schatz, G.H., Brock, H. and Holzwarth, A.R. (1988) Biophys. J. 54, 397–405.
28 Schlodder, E. and Brettel, K. (1988) Biochim. Biophys. Acta 933, 22–34.
29 Takahashi, Y., Hansson, O., Mathis, P. and Satoh, K (1987) Biochim. Biophys. Acta 893, 49–59.
30 Berthold, D.A., Babcock, G.T. and Yocum, C.F. (1981) FEBS Lett. 134, 231–234.
31 Ford, R.C. and Evans, M.C.W. (1983) FEBS Lett. 160, 159–164.
32 Blasie, J.K., Erecinska, M., Samuels, S. and Leigh, J.S. (1978) Biochim. Biophys. Acta 501, 33–52.
33 Rutherford, A.W., Paterson, D.R. and Mullet, J.E. (1981) Biochim. Biophys. Acta 635, 205–214.
34 Den Blanken, H.J., Hoff, A.J. Jongenelis, A.P.J.M. and Diner, B.A. (1983) FEBS Lett. 157, 21–27.
35 Prince, R.C., Crowder, M.S. and Bearden, A. (1980) Biochim. Biophys. Acta 592, 323–337.
36 Thurnauer, M.C. and Norris, J.R. (1977) Chem. Phys. Lett. 47, 100–105.
37 Durrant, J.R., Giorgi, L.B., Barber, J., Klug, D.R. and Porter, G. (1990) Biochim. Biophys. Acta 1017, 167–175.
38 Van Kan, P.J.M., Otte, S.C.M., Kleinherenbrink, F.A.M., Nieveen, M.C., Aartsma, T.J. and Van Gorkom, H.J. (1990) Biochim. Biophys. Acta 1020, 146–152.
39 Tetenkin, V.L., Gulyaev, B.A., Seibert, M. and Rubin, A.B. (1989) FEBS Lett. 250, 459–463.
40 Braun, P., Greenberg, P.M. and Scherz, A. (1990) Biochemistry 29, 10376–10387.
41 Okamura, M.Y., Satoh, K., Isaacson, M.A. and Feher, G. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. 1, pp. 379–381, Martinus Nijhoff, Dordrecht.
42 Renger, G. (1991) in Topics in Photosynthesis (Barber, J., ed.), Vol. 11, Elsevier, Amsterdam, in press.
43 Knapp, E.W., Fischer, S.F., Zinth, W., Sander, M., Kaiser, W., Deisenhofer, J. and Michel, H. (1985) Proc. Natl. Acad. Sci. USA 82, 8463–8467.
44 Scherer, P.O.J. and Fischer, S.F. (1987) Biochim. Biophys. Acta 891, 157–164.
45 Lösche, M., Feher, G. and Okamura, M.Y. (1988) in The Photosynthetic Bacterial Reaction Center Structure and Dynamics (Breton, J. and Verméglio, A., eds.), pp. 151–164, Plenum Press, New York.
46 Lösche, M., Satoh, K., Feher, G. and Okamura, M.Y. (1988) Biophys. J. 53, 270a.
47 Pearlstein, R.M. (1982) in Photosynthesis: Energy Conversion by Plants and Bacteria (Govindjee, ed.), pp. 293–330, Academic Press, New York.