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ORIENTATION OF THE PRIMARY DONOR IN ISOLATED PHOTOSYSTEM II REACTION CENTERS STUDIED BY ELECTRON PARAMAGNETIC RESONANCE

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Isolated photosystem II (PS II) reaction center complexes were partially dried on polyester (mylar) sheets. Using electron paramagnetic resonance (EPR) measurements of the cytochrome b_{559} that is present in the PS II reaction center, we found that the isolated complexes were oriented on the mylar with the same geometry as that shown when oriented in the natural membrane. From the orientation dependence of the EPR signal from ^{3}P680, the triplet state of the primary donor chlorophyll, we concluded that the plane of the chlorophyll macrocycle was oriented parallel to the mylar sheet and therefore parallel to the plane of the natural membrane. This is different from the situation in purple bacteria where the analogous primary bacteriochlorophyll donor is perpendicular to the membrane plane.

RESULTS

PS II reaction center complexes were isolated from spinach chloroplast membranes as described previously (1). The isolated reaction centers were painted onto mylar sheets and dried in a 90% humidity argon atmosphere for 48 h at 4°C in darkness. EPR spectra of the dried films showed signals characteristic of cytochrome b_{559} in its low potential oxidized form. Fig. 1 shows that the two low-spin haem signals ($gz = 2.97$, $gy = 2.2$) were orthogonal, with the gz signal being maximum when the mylar sheet was parallel to the magnetic field. This orientation dependence is the same as that found for this cytochrome in oriented membrane preparations and has been interpreted previously as indicating that the haem plane is perpendicular to the membrane plane (reviewed in reference 2). We therefore concluded that, after being dried, the isolated reaction center complexes become ordered in two dimensions with the same geometry as in the native membrane. This may be due to the alignment of adjacent hydrophobic and hydrophyllic regions of these membrane-spanning, intrinsic proteins.

When the isolated PS II reaction centers were reduced with sodium dithionite in darkness, an EPR signal at g 2.0045, ($\Delta H \simeq 9$ G) was induced. This signal is attributed to the semiquinone form of the primary plastoquinone acceptor, Q_A, in the absence of the characteristic interaction with iron (reviewed in reference 2). This signal showed little anisotropy.

When reduced PS II reaction centers are illuminated at low temperature, the following photochemistry takes place:

$$P680\ Ph\ Q_A^- \xrightarrow{light} P680^+\ Ph^-\ Q_A^- \longrightarrow {}^3P680\ Ph\ Q_A^-,$$

where Ph is pheophytin, the primary electron acceptor and ^{3}P680 is the unusual spin-polarized triplet state of P680 formed by recombination of the radical pair, $P680^+\ Ph^-$.

^{3}P680 was detected by EPR in the oriented PS II reaction centers (Fig. 2). The ^{3}P680 signal was orientation dependent with the outer Z peaks showing clear maxima when the mylar was perpendicular to the magnetic field, while the X and Y peaks showed less well-marked maxima when the mylar was parallel to the magnetic field. The Z peak is associated with the axis perpendicular to the chlorophyll macrocycle (3). Thus the plane of the chlorophyll macrocycle is parallel to the mylar sheet. The data on the cytochrome showed that the isolated reaction centers were oriented on the mylar with the same geometry as in the native membrane. Therefore it is concluded that the plane of the chlorophyll macrocycle of ^{3}P680 is oriented parallel to the plane of membrane. This agrees with the conclusion obtained from similar but less well-resolved data using oriented PS II membranes. (2).

This conclusion is of interest in two respects. First, it indicates that the extremely rapid electron transfer reaction between P680 and Ph occurs between planar mole-

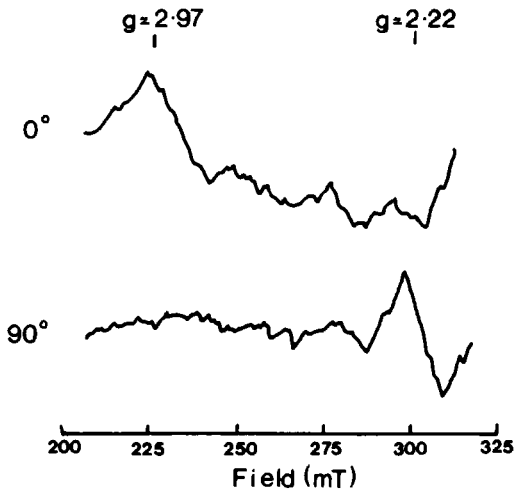

FIGURE 1 EPR spectra of cytochrome b_{559} in isolated PS II reaction centers when partially dried on mylar. Instrument settings were as follows: temperature, 15 K; microwave power, 15 dB down from 200 mW; modulation amplitude, 20 G. Spectra are shown which were obtained when the mylar sheets were parallel (0°) and perpendicular (90°) to the magnetic field.

cules that are perpendicular to each other (see reference 2). This is also the case in the analogous purple bacteria reaction center (4). Secondly, the orientation of the PS II primary donor chlorophyll in the plane of the membrane is different from the situation in purple bacteria where the primary donor bacteriochlorophyll is oriented almost perpendicular to the membrane plane. This represents a significant structural difference between the reaction centers of PS II and purple bacteria. Such differences are not unexpected, because the operating redox potential of P680, as an oxidant of water, is estimated to be at least 0.5 V more positive than that of purple bacteria.

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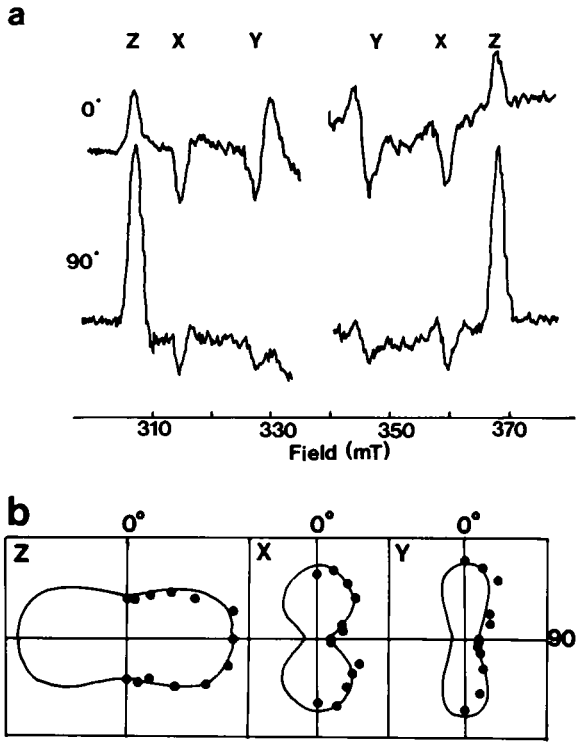

FIGURE 2 The orientation dependence of the P680 triplet in isolated PS II reaction centers. The sample, which was partially dried on mylar sheets, was submerged in sodium dithionite (20% in 200 mM glycine pH 9.0) under an 0_2 free argon atmosphere for 5 min in darkness at 22°C. Instrument settings were as follows: temperature, 4.2 K; microwave power 35 dB down from 200 mW, modulation amplitude 20 G. Spectra were recorded under illumination using white light from an 800 watt projector. (*a*) EPR spectra recorded when the mylar sheets were parallel (0°) and perpendicular (90°) to the magnetic field. (*b*) Polar plots of the *ZXY* features of the triplet signal.

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BILAYER PENETRATION BY MEMBRANE-ASSOCIATED PROTEINS

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The influenza virus consists of a core complex of protein and RNA that becomes coated with a lipid envelope as the maturing virus buds from the host cell (1). This envelope contains two major intrinsic proteins; one of which, hemagglutinin (HA), is known to form trimeric aggregates (2) appearing as characteristic spikes in electron micrographs

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