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ORIENTATION OF THE PRIMARY QUINONE OF BACTERIAL PHOTOSYNTHETIC REACTION CENTERS CONTAINED IN CHROMATOPHORE AND RECONSTITUTED MEMBRANES

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The orientation of the reaction center bacteriochlorophyll dimer, $(BChl)_2$, and primary quinone, Q_I, has been studied by EPR in chromatophores of *Rhodopseudomonas sphaeroides* R26 and *Chromatium vinosum* and in the reconstituted membrane multilayers of the isolated *Rps. sphaeroides* reaction center protein. The similarity in the angular dependence of the $(BChl)_2$ triplet and $Q_I^- Fe^{2+}$ signals in the chromatophore and reconstituted reaction center membrane multilayers indicates that the reaction center is similarly oriented in both native and model membranes. The principle magnetic axes of the $(BChl)_2$ triplet are found to lie with the *x* direction approximately parallel to the plane of the membrane surface, and the *z* and *y* directions approx. 10–20° away from the plane of the membrane surface and membrane normal, respectively. The $Q_I^- Fe^{2+}$ signals are found to have the *g* 1.82 component positioned perpendicular to the plane of the membrane surface, with an orthogonal low-field transition (at *g* 1.68 in *Rps. Sphaeroides* and at *g* 1.62 in *C. vinosum*) lying parallel to the plane of the membrane surface. The orientation of Q_I was determined by the angular dependence of this signal in Fe^{2+}-depleted reaction center reconstituted membrane multilayers, and it was found to be situated most likely with the plane of the quinone ring perpendicular to the plane of the membrane surface.

Introduction

The primary light-initiated electron-transfer reactions of the bacterial photosynthetic reaction center provide a special opportunity to investigate structural as well as kinetic and thermodynamic parameters responsible for biological electron transfer. A salient feature of these early photosynthetic electron-transfer reactions is the lack of a dominating temperature sensitivity [1–6] and, therefore, the probability that the reactions proceed without large scale rearrangements of reactants. The most recent theories to account for these data have invoked a mechanism of vibronically coupled electron tunnelling [7,8] between the redox centers that are held at specific points within the protein matrix of the reaction center.

The light-activated reactions are considered to proceed as follows (see Refs. 9–11 for recent reviews). The initial light-driven electron transfer occurs, possibly via a monomeric bacteriochlorophyll (BChl) [13], between a bacteriochlorophyll dimer, $(BChl)_2$, and a bacteriopheophytin (BPh) to form $(BChl)_2^+ BPh^-$. The BPh^- so formed then transfers an electron in a half-time of 100–200 ps to a quinone (designated Q_I) to form $(BChl)_2^+ BPhQ_I^-$. In isolated reaction centers, an electron can return from Q_I^- back to $(BChl)_2^+$ in a half-time of 20 ms (below 100 K) to 60 ms (300 K). If Q_I is removed or is already reduced before activation, an electron returns from BPh^- to $(BChl)_2^+$ in a half-time of 10 ns (300 K) to 30 ns (below 100 K). With lowering temperatures this reaction increasingly produces a triplet state of $(BChl)_2$ which below 20 K is detectable by EPR as a remarkable spin-polarized triplet signal.

Previously, we attempted to place limits on the distances between some of the reaction center redox

components by examining the strengths of the magnetic interaction between these components in reaction centers of *Rhodopseudomonas sphaeroides* and *Chromatium vinosum* [14–16]. In addition, in *C. vinosum* we have also examined the orientation and distance between the reaction center-associated cytochromes *c* and $(BChl)_2$ [15]. These studies indicated that in both the millisecond reactions between Q_I^- and $(BChl)_2^+$ and between ferrocytochrome *c* and $(BChl)_2^+$, the redox groups are separated by edge-to-edge distances of greater than 13–15 Å [15,16], while in the case of the 150–250 ps reaction between BPh^- and Q_I, the two redox groups are close enough to experience a significant orbital overlap as exhibited by a magnetic exchange interaction [14], reflecting edge-to-edge separation perhaps of the order of 10 Å [16,17].

The angular orientation of the reaction center bacteriochlorophylls and bacteriopheophytin has been examined by optical linear dichroism measurements in *Rps. spaeroides* chromatophore membrane multilayers [18,19], in stretched gelatin films containing isolated reaction centers [19,20], and by optical photoselection in solution [21]. The orientation of $(BChl)_2$ has also been investigated by examination of the $(BChl)_2$ triplet EPR signal in membrane multilayers of *Rhodopirillum rubrum* [22]. These studies have shown that both the $(BChl)_2$ triplet in-plane EPR absorptions and the optical Q_x and Q_y transitions of $(BChl)_2$ lie in a plane which is nearly perpendicular to the plane of the membrane.

In this paper, we have examined by EPR the orientation of the Q_I molecule in reconstituted membrane multilayers of the isolated *Rps. sphaeroides* reaction center protein, as well as in the native chromatophore membranes of *Rps. sphaeroides* and *C. vinosum*. The reaction center Q_I is located close to a high-spin Fe^{2+} which induces a large anisotropy in the Q_I^- EPR signal [9–11]. Unfortunately, the correlation between the anisotropic $Q_I^-Fe^{2+}$ signal and the molecular structure is not known, preventing direct determination of the Q_I orientation from this signal. The problem was circumvented by utilizing the smaller but better understood [22–24] anisotropy of Q^- in reconstituted membrane multilayers containing Fe^{2+}-depleted isolated reaction centers. We show that reaction centers, both normal and Fe^{2+} depleted, are reconstituted in phopholipid membranes with an orientation similar to that in the chromatophore membrane, as judged by similarities in the orientation of the $(BChl)_2$ triplet and $Q_I^-Fe^{2+}$ signals. In the Fe^{2+}-depleted reaction centers, we find that the plane of the aromatic ring of the ubiquinone is likely to be positioned normal to the plane of the membrane surface.

Materials and Methods

Reaction center preparations. The photosynthetic bacteria *C. vinosum* and *Rps. sphaeroides* were grown anaerobically in the light with succinate as the sole carbon source as previously described [25]. Deuterated cells were grown for several transfers on the same medium, with the exception that water was replaced by ^2H_2O. Chromatophores were prepared by passing the cells through a French pressure cell followed by the usual differential centrifugation procedures.

Reaction centers from *Rps. sphaeroides* R26 were prepared using the detergent lauryldimethylamine *N*-oxide (LDAO) essentially as described by Clayton and Wang [26]. The isolated reaction centers were found to contain only one ubiquinone per reaction center [11,12] as is indicated by the following observations. First, only a single kinetic decay parameter of the light-induced $(BChl)_2^+Q_I^-Fe$ state is observed with an approx. 60 ms half-time [27–29]; this contrasts with the decay of the $(BChl)_2^+$ state from secondary quinones which takes place on the seconds time scale [27–29]. Second, following a train of saturating flashes spaced 40 ms apart, a single cytochrome *c* oxidation ($t_{1/2} < 0.1$ ms) occurs only on the first flash when reaction centers are incubated in the presence of a 50-fold excess of ferrocytochrome *c* (see Ref. 28 and 29). The lack of reoxidation of Q_IFe by secondary quinones permits only a single turnover of the reaction center. For further discussions on the changes in the light-induced reactions with reaction centers containing more than one quinone, see Refs. 27–31.

Iron-depleted reaction center preparations. Reaction centers were depleted of iron in two ways. First, by treatment with KSCN [32]. Reaction centers (100 μM) in 0.05% LDAO, 10 mM Tris-HCl, pH 8.0, were dialyzed for 2 h at room temperature against 2 M KSCN, 1 mM *o*-phenanthroline, 10 mM Tris-HCl, pH 8.0, followed by overnight dialysis at 6°C against

10 μM EDTA, 0.05% LDAO and 10 mM Tris-HCl, pH 8.0.

Alternatively, reaction centers were dialyzed for 1 h at room temperature against 0.75 M $LiClO_4$, 1 mM *o*-phenanthroline, 50 mM $CaCl_2$, 10% ethanol, 0.05% LDAO, 10 mM Tris-HCl, pH 8.0, followed by the same overnight dialysis as described above. In addition to depletion of Fe^{2+}, this procedure has been shown to remove the reaction center H protein subunit [33].

In both cases, iron-depleted reaction centers were re-chromatographed on a DE-52 column to remove reaction centers destroyed by the treatments.

Reaction center/membrane reconstitution. The reconstitution of reaction center into phospholipid vesicles was accomplished by taking advantage of the chemical lability of the detergent LDAO at low redox potentials [34]. Reaction centers in 0.05% LDAO, 10 mM Tris-HCl, pH 8.0, with a protein purity indicated by a 280 nm/800 nm optical absorbance ratio of 1.2–1.3 were diluted to a concentration of 3 μM, in a suspension of 450 μM phosphatidylcholine (isolated from egg yolks according to the method of Singleton et al. [35]) and 10 mM Tris-HCl, pH 8. The reaction center/phosphatidylcholine suspension was kept at room temperature in a vessel equipped for sonication and anaerobic redox potentiometry under an argon atmosphere. The redox potential of the solution was lowered to an E_h of −250 mV for 5 min to permit the complete reduction of LDAO [34]. Vesicles were formed by sonicating (Branson Microtip, low power at 10 kHz) the reduced solution with three 20-s periods, spaced 1 min apart. The reduced solution was then dialyzed against 10 mM Tris-HCl, pH 8.0 for 24–40 h to remove the reduction products [34].

Preparation of oriented multilayers. Multilayers were prepared by sedimentation (70 000 × *g* for 3 h) of the reaction center/phosphatidylcholine dispersion onto a Mylar support, using a lucite sedimentation cell adapted to a Beckman swinging-bucket SW 25.1 rotor as previously described [34]. The resulting pellet had a 1 cm diameter and contained 9 nmol reaction center. The multilayer pellet was partly dehydrated for 24–40 h at 6°C in a 90–95% humidity chamber maintained by a saturated aqueous $ZnSO_4$ solution.

A sample was prepared for EPR analysis by slicing the partly dehydrated multilayers and Mylar support into 3-mm strips, which were aligned in an EPR tube, purged with helium gas, sealed and then frozen in liquid nitrogen.

Multilayer samples which contained bound cytochrome *c* as an electron donor to $(BChl)_2$ were also prepared according to these procedures, except that 50 μM cytochrome *c* was present in the reaction center/phosphatidylcholine dispersion during the sedimentation.

Reduction of Q_I and generation of the $(BChl)_2$ triplet signal in membrane multilayers. The QFe^{2+} complex undergoes one-electron reduction, which in the isolated *Rps. sphaeroides* reaction center has a pH-independent midpoint of −50 mV [9], giving rise to an anisotropic EPR absorption centered at *g* 1.82 in the reduced state [9]. In chromatophores the QFe^{2+} complex shows a pH-dependent midpoint which has a p*K* of 8–10 and a midpoint potential of −160 to −200 mV in the various species [9]. The stability of the reduced $Q^{-}Fe^{2+}$ species permits its generation by wetting the multilayers with a dithionite solution followed by freezing in liquid nitrogen.

However, with the KSCN-treated, iron-depleted reaction centers, chemical reduction of Q_I was found not to generate an appreciable amount of the $Q_I^{-\cdot}$ free radical species, and equilibrium redox titrations of this quinone acceptor are suggestive of a two-electron reduction. Instead, a stable $Q_I^{-\cdot}$ free radical was trapped in the light by using the rapid (less than 0.1 ms) electron transfer from cytochrome *c* to $(BChl)_2^{+\cdot}$ to outcompete the $t_{1/2}$ = 140 ms back reaction from $Q_I^{-\cdot}$ to $(BChl)_2^{+\cdot}$ in the iron-depleted reaction centers:

$$\text{ferrocytochrome } c\ (BChl)_2Q_I \xrightarrow{h\nu} \text{ferrocytochrome } c\ (BChl)_2^{+\cdot}Q_I^{-\cdot} \xrightarrow[<0.1\text{ ms}]{} \text{ferricytochrome } c\ (BChl)_2Q_I^{-\cdot}$$

This was accomplished in the reaction center multilayers containing bound cytochrome *c* by moistening the multilayers with a 5 mM ascorbate solution prior to placing them in an EPR tube, illuminating the same for 1 s followed by rapid freezing in liquid nitrogen.

It has been previously shown that reaction centers are incorporated asymmetrically into the phosphatidylcholine membranes by the LDAO reduction tech-

nique, having greater than 85% of $(BChl)_2$ accessible to reduction by externally added cytochrome *c* [34]. This asymmetry is also reflected in the ascorbate-treated reaction center/cytochrome *c* multilayers. The optical absorbance changes measured at 550 nm following a series of saturating flashed showed a single rapid cytochrome *c* oxidation on the first flash, and the redox changes of $(BChl)_2$ monitored at 605 nm showed a rapid oxidation with a re-reduction on a commensurate time scale to the cytochrome *c* oxidation following the first flash. Successive flashes yielded no further $(BChl)_2$ oxidation on the millisecond time scale, reflecting the inhibition of the normal photochemistry by prior reduction of the quinone acceptor.

The optical changes arising from the anionic $Q_I^{-\cdot}$ in the visible region were monitored by treating the reaction center multilayers without cytochrome *c*, but with 1 mM 2,3,5,6-tetramethylphenylenediamine, 5 mM ascorbate as an electron donor to $(BChl)_2$. These samples showed a light-induced spectrum which matched that of $Q_I^{-\cdot}$ reported previously, having an absorbance peak at 450 nm [30–31]. Re-oxidation of $Q_I^{-\cdot}$ was blocked by the prior reduction of $(BChl)_2^{+}$ by the diaminodurene, and $Q_I^{-\cdot}$ is seen to decay with a half-time of 15–30.

The $(BChl)_2$ triplet [9] was generated by illumination at temperatures below 15 K in multilayer samples in which Q_I was previously reduced, either by chemical reduction or by the photochemical trapping procedures described above.

Spectrometry. EPR spectra were recorded with a Varian E109 spectrometer equipped with the E-231 cavity operating in the TE_{201} mode. Temperature was controlled with a flowing helium (Air Products) or nitrogen cryostat (Varian). Temperature was monitored with a calibrated carbon resistor (below 20 K) or thermocouple (above 20 K) placed approx. 1.5 cm below the sample in the gas flow. Reliability of the temperature measurement was confirmed by finding the expected Curie Law dependence of the copper signal in dilute $CuSO_4$/EDTA solutions. Absolute temperature corrections were made by placing a second calibrated carbon resistor in a blank sample EPR tube, and comparing the measurements of the two resistors.

Light-induced optical spectra were measured on a Johnson Foundation dual-wavelength spectrophotometer, used in a single mode with xenon (6 μs) flash. Multilayer samples formed on either Mylar or quartz supports were tilted at 45° to both the xenon flash and measuring beam path.

Results

The $(BChl)_2$ triplet signal

The highly anisotropic EPR signal of the $(BChl)_2$ triplet/biradical [36,37] provides a convenient assay to compare the relative orientation of $(BChl)_2$ in the different reaction center reconstituted membranes with that of the chromatophore membrane.

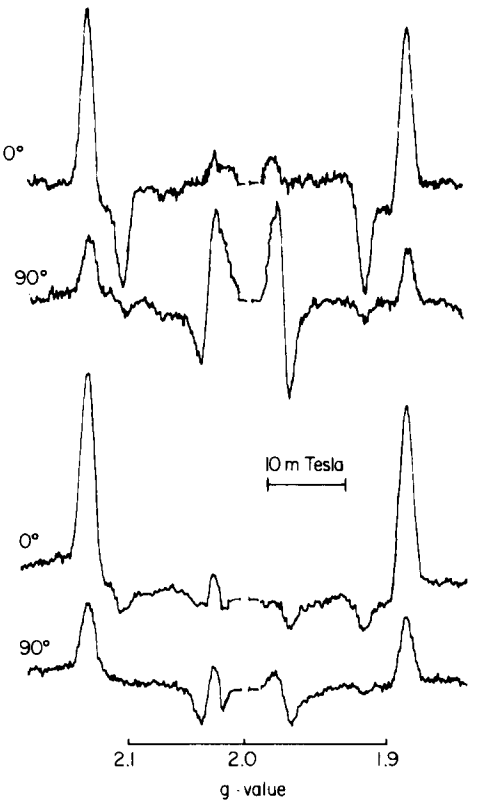


Fig. 1. The light-induced $(BChl)_2$ triplet EPR signal in reaction center reconstituted membrane multilayers. The light-minus-dark spectra were recorded at 8 K with the magnetic field aligned parallel to the plane of the membrane surface (0°) and perpendicular to the plane of the membrane surface (90°). The spectra were recorded with spectrometer gain of $1.25 \cdot 10^4$ and $6.2 \cdot 10^3$ for the untreated (upper traces) and KSCN-treated (lower traces) reaction center multilayers, respectively, and with 16 G modulation amplitude and 1 mW of microwave power.

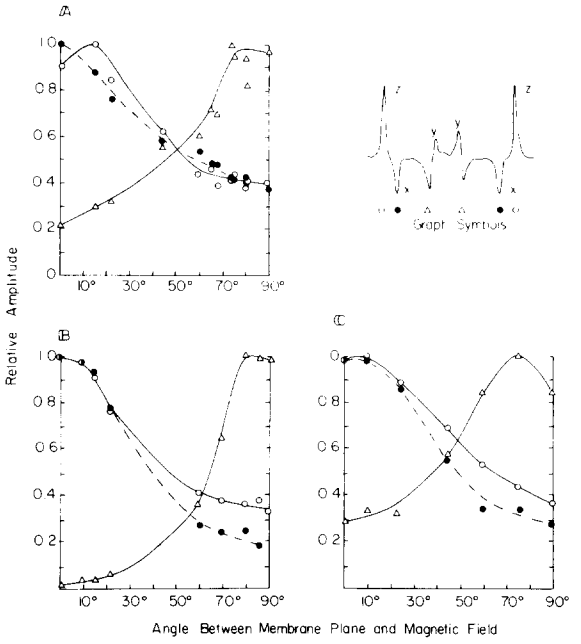


Fig. 2. Angular dependence of the $(BChl)_2$ triplet EPR signal in *Rps. sphaeroides* chromatophores and reconstituted membrane multilayers. Spectra were recorded as described in Fig. 1, with the membrane at various angles to the magnetic field. (A) Chromophores, (B) untreated and (C) KSCN-treated reaction center/phosphatidylcholine vesicles.

This measurements provides an indication of the similarity in the orientation of the reconstituted reaction center proteins.

The $(BChl)_2$ triplet signal shows three pairs of absorption/emission transitions [36,37], labeled by convention, Z, X and Y for the outer to inner pairs. These correspond to the alignment of the magnetic field along the principle magnetic directions of the dimer. Fig. 1 shows the light-induced triplet signal in reconstituted reaction center multilayers and in the KSCN-treated, Fe^{2+}-depleted reaction center membranes. The light-minus-dark difference spectra are recorded with the Mylar support and the plane of the membrane surfaces either parallel to the applied magnetic field (0°) or perpendicular to the magnetic field (90°). The $(BChl)_2$ triplet signal in both the reaction center and Fe^{2+}-depleted reaction center membrane multilayers shows a similar sensitivity to the angular alignment in the magnetic field, although by comparison in the iron-depleted reaction centers the maximal intensities of the X and Y transitions are much less intense relative to the transitions in the *z* direction. The latter alteration in relative intensities of the three components of the $(BChl)_2$ triplet signal for the KSCN-treated reaction center is also seen in the detergent-solubilized, randomly oriented frozen solution, and may reflect an increased heterogeneity in the rhombicity of $(BChl)_2$ in the Fe^{2+}-depleted samples.

The variations in the relative intensities of the X, Y and Z transitions for the $(BChl)_2$ triplet signal are plotted in Fig. 2 as the angle between the plane of the membrane surface and the magnetic field is rotated from 0 to 90°. The results are taken from measurements recorded from chromatophore membrane multilayers as well as in reaction center and iron-deplete reaction center reconstituted membrane multilayers. These data show that $(BChl)_2$ is situated in a similar orientation with respect to the plane of the membrane in both the chromatophore membrane and the reconsitituted reaction centers, with the *X* direction of the $(BChl)_2$ triplet lying approximately parallel to the plane of the membrane, while the *Z* and *Y* directions are found to lie approx. 10–20° away from the plane of the membrane surface and membrane normal, respectively. This orientation of the $(BChl)_2$ triplet in *Rps. sphaeroides* chromatophores and reconstituted reaction centers is similar to the orientation determined previously in chromatophores of *R. rubrum* [22].

The Q_I-Fe^{2+} signal

The orientation of the reaction center in chromatophores and reconstituted membranes can also be compared by examining the relative orientation of the reduced Q_IFe complex, which shows an anisotropic EPR absorption centered at *g* 1.82. Fig. 3 shows the Q_I-Fe^{2+} EPR absorption in reaction center reconstituted phosphatidylcholine multilayers, chromatophores of *Rps. sphaeroides,* and in chromatophore multilayers of *C. vinosum,* recorded with the membrane plane at 90 and 0° to the magnetic field. The angular dependence of the components of the Q_I-Fe^{2+} signal in *Rps. sphaeroides* chromatophore and reconstituted reaction center multilayers is shown in Fig. 4. The Q_IFe^{2+} complex was reduced by illumination at room temperature in the presence of ferrocytochrome *c* in the reconstituted reaction

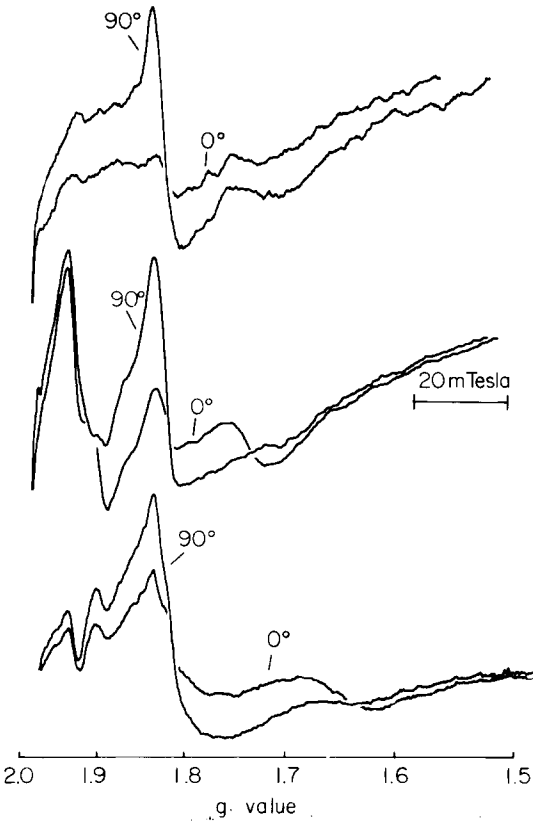


Fig. 3. The $Q_I^{-}Fe^{2+}$ EPR signals in *Rps. sphaeroides* chromatophores (center), reaction center reconstituted membrane multilayers (top) and in *C. vinosum* chromatophore multilayers (bottom). Spectra were recorded at 8 K using 25 G of modulation amplitude and 20 mW of microwave power. Angles refer to those between the plane of the membrane and the magnetic field.

center membranes as described in Materials and Methods, and by soaking the multilayers with a dithionite solution in the chromatophore membrane.

The g 1.82 component of the $Q_I^{-}Fe^{2+}$ signal is found to be most intense when the magnetic field is perpendicular to the membrane plane, while the 'g_x' or high-field component of the signal, located at g 1.68 in *Rps. sphaeroides* and g 1.62 in *C. vinosum*, is found to be most intense when the magnetic field lies in the plane of the membrane; although in the *Rps. spheroides* reconstituted reaction centers the g 1.68 component was not reproducibly resolvable. These results suggest that the g 1.82 and the g 1.68 or g 1.62 components represent orthogonal EPR transitions, and that the $Q_I^{-}Fe^{2+}$ component is similarly oriented in both the *Rps. sphaeroides* chromatophore and reaction reconstituted membrane as well as in *C. vinosum* chromatophore, where the Q_I is a menaquinone (a naphthoquinone) [38] in contrast to the ubiquinone (a benzoquinone) of *Rps. sphaeroides*. Without knowing the correlation between the EPR transitions and the molecular structure of the quinone-Fe^{2+}, these results cannot be extrapolated to predict the absolute orientation of the $Q_I^{-}Fe^{2+}$ complex.

Q_I in the absence of an interaction with Fe^{2+}

With X-band (9 GHz) EPR the Q_I^{-} radical, formed centers by illumination in the presence of ferrocytochrome *c*, appears as a nearly symmetric 8 G wide signal centered at g 2.005 [23,24]. The K-band

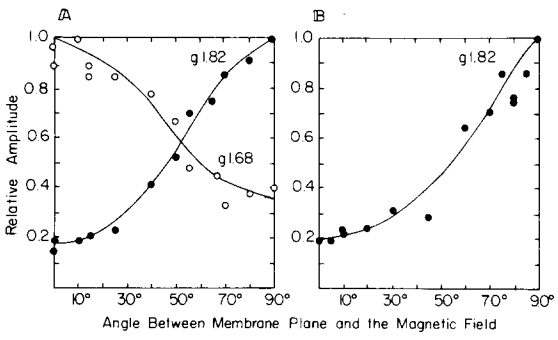


Fig. 4. Angular dependence of the $Q_I^{-}Fe^{2+}$ EPR signal in *Rps. sphaeroides* membrane multilayers. Spectra were recorded with conditions as described in Fig. 3. (A) Chromophores, (B) reaction center/phosphatidylcholine vesicles.

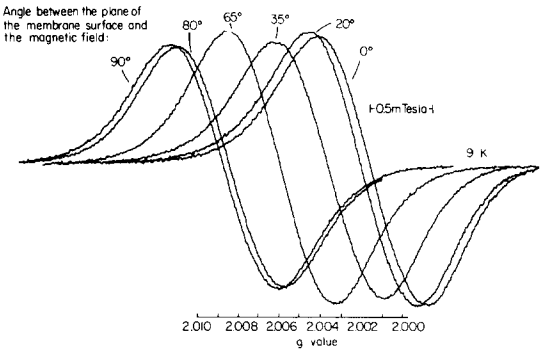


Fig. 5. Angular dependence of the Q_I^{-} EPR signal in KSCN-treated reaction center membrane multilayers. Spectra were recorded at 9 K using 1 μW of microwave power and 3.2 G of modulation amplitude.

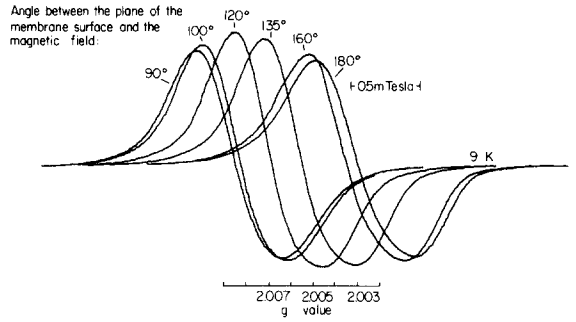


Fig. 6. Angular dependence of the Q_I^- signal in KSCN-treated, deuterated reaction centers in membrane multilayers. Spectra were recorded as in Fig. 5, except with a 1 G modulation amplitude.

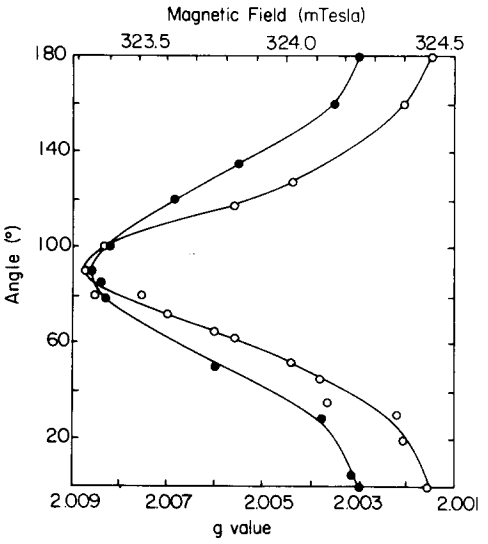


Fig. 7. Angular dependence in the position of the Q_i^- EPR signal in KSCN-treated reaction center reconstituted membrane multilayers. Data points were takem from spectra as shown in Figs. 5 and 6, and represent the zero-crossing points of the spectra recorded at various angles between the plane of the membrane surface and the magnetic field. (●———●) Deuterated reaction centers, (○———○) protonated reaction centers.

(35 GHz) spectra and spectra of deuterated reaction centers recorded with X-band EPR show the Q_I^- free radical (and in vitro ubisemiquinone anion) to appear as a nearly axial spin system, having the average $g_{||}$ absorptions at approx. g 2.0055 and $g_\perp$ at g 2.0023, which corresponds to approx. 5 G of electronic g value anisotropy at X-band EPR [23,24]. The free electron g 2.0023 value can be expected to be associated with a direction normal to the quinone ring, while an EPR absorption correspondingly to the oxygen-oxygen axis can be expected to be associated with the largest g value [22,39]. These assignments permit determination of the quinone orientation if the direction of the g tensor with respect to the membrane plane can be measured.

Fig. 5 shows the Q_I^- EPR signal recorded at 9 K in KSCN-treated, iron-depleted reaction center membrane multilayers. The signal was formed by illumination at room temperature in the presence of ferrocytochrome c (see Materials and Methods), The position of the EPR absorption for Q_I shows a strong dependence upon the angular orientation of the membrane multilayer in the magnetic field. The EPR absorption is seen to have the lowest g value when the magnetic field is aligned parallel to the plane of the membrane surface, while the highest g value absorption occurs with the magnetic field perpendicular to the membrane surface. A plot of the position of the Q_I^- absorption at 9 K as the angle between the membrane plane and the magnetic field is rotated from 0 to 180° is shown in Fig. 7. The 11–12 G shift resonance position of the Q_I^- in the KSCN-treated reaction center multilayers far exceeds the approx. 5 G maximum expected from the previously determined g value anisotropy. This anomaly could in part arise from the slow spin relaxation of the Q_I^- at 9 K, as the signal is partly unsaturated with the 1 μW microwave power used to record these spectra and exhibits some lineshape dependence upon microwave power. Anomalies due to rapid passage effects were tested by recording the spectra with low modulation frequency (35 Hz) and amplitude (0.5 G), and by recording spectra in both forward and reverse directions, which were found not to alter appreciably either the lineshape or resonance position.

Temperature, however, had a marked effect on the angular dependence of the Q_I^- EPR absorption. This is shown in Fig. 8, where the EPR absorption of Q_I^- is recorded at various temperatures with the membrane plane parallel and perpendicular to the magnetic field. As the temperature is raised the positions of the EPR absorptions at 90 and 0° move toward, and essentially coalesce at temperatures above 100 K, a g value of 2.005. The maximal difference in the position of the EPR absorptions remains

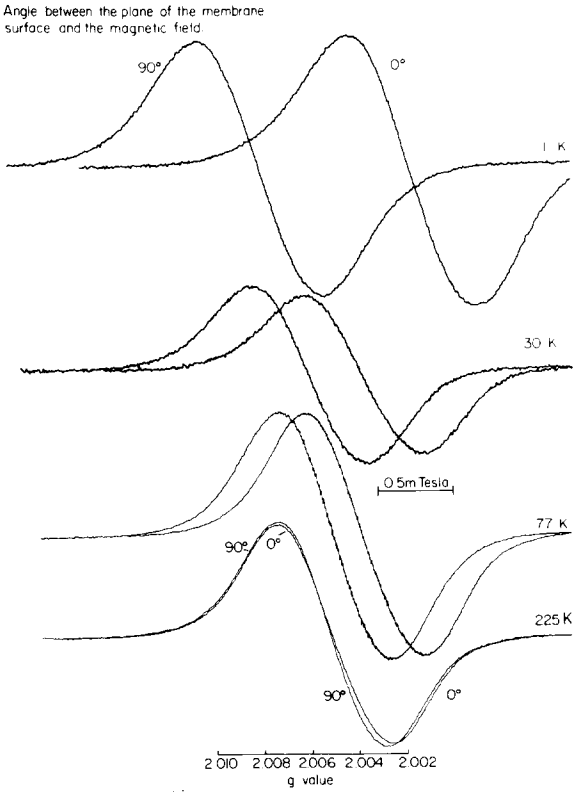


Fig. 8. Effect of temperature on the angular dependent shift in the resonance position of the Q_I^- signal in KSCN-treated center reconstituted membrane multilayers. The maximum shift in the resonance position remains between the parallel (0°) and perpendicular (90°) alignment of the membrane multilayers in the magnetic field for all temperatures. Spectra were recorded with a 3.2 G modulation amplitude, with the following spectrometer gains and microwave powers: $6.3 \cdot 10^3$, 1 μW (11 K); $8 \cdot 10^3$, 1 μW (30 K); $3.2 \cdot 10^3$, 10 μW (77 K); $2.5 \cdot 10^3$, 100 μW (225 K).

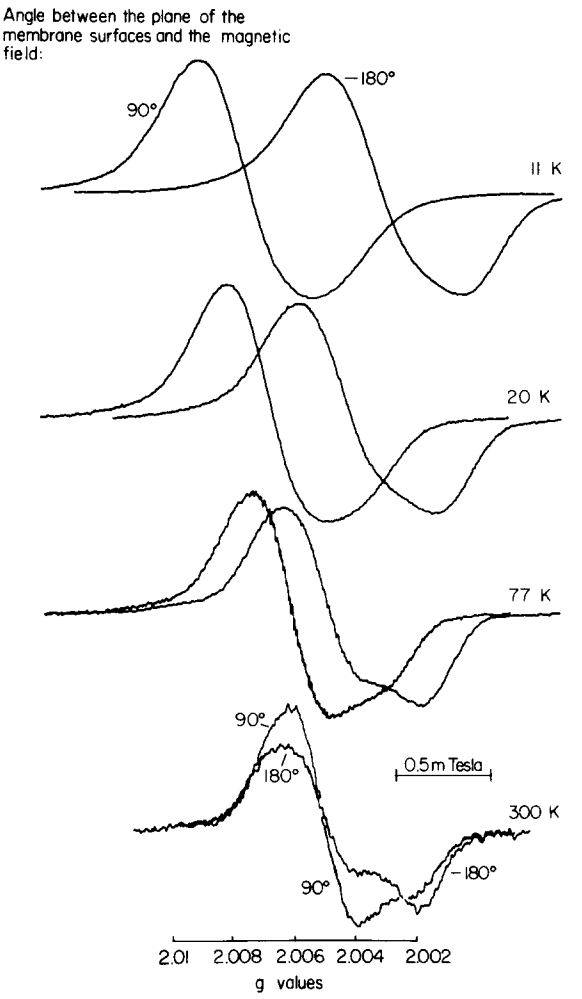


Fig. 9. Effect of temperature on the angular dependent shift in the resonance position of the Q_I^- signal in KSCN-treated, deuterated reaction center reconstituted membrane multilayers. Spectra were recorded as in Fig. 8, except with 1 G modulation amplitude and with the following gains and microwave powers: $2.5 \cdot 10^3$, 1 μW (11 K); $4 \cdot 10^3$, 1 μW (20 K); $8 \cdot 10^3$, 1 μW (77 K); $1.25 \cdot 10^4$, 5 μW (300 K).

between signals recorded with the magnetic field parallel and perpendicular to the plane of the membrane, and the residual angle-dependent change in lineshape shown at 225 K in Fig. 8 remains at room temperature. These temperature-dependent shifts can be reversed by lowering the temperature. The temperature dependence of the maximal resonance shift, measured by the difference in the zero-crossing points at 0 and 90°, is shown in Fig. 10 on an Arrhenius plot.

Deuterated Q_I in the absence of an interaction with iron

The hyperfine broadening of the native Q_I^- signal obscures much of the g value anisotropy, especially at the higher temperatures in the multilayer samples. Multilayers of deuterated reaction centers were prepared in order to examine more closely the temperature-dependent shift in the g value and the angular-dependent shift in the Q_I^- EPR absorption.

Fig. 6 shows the angular dependence of the Q_I^- signal in the KSCN-treated, deuterated reaction center membrane multilayers, and a plot of the position of the EPR absorption vs. angle is shown in

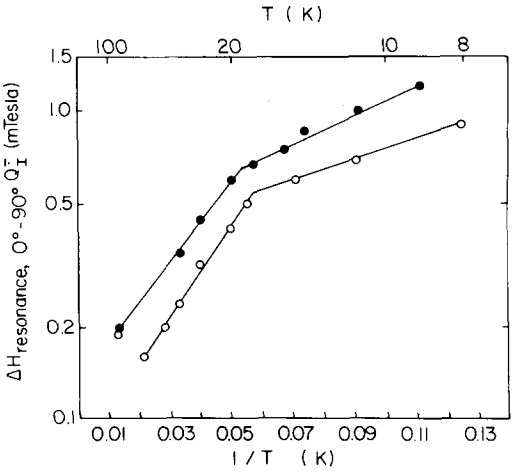


Fig. 10. Plot of the temperature dependence of the maximal resonance shift of the Q_I^- signal. Data were taken from the zero-crossing points of spectra recorded with membrane multilayers aligned parallel and perpendicular to the magnetic field as described in Figs. 8 and 9. The slopes represent apparent activation energies of 20.6 and 7.4 cm^{-1} for the protonated sample and 23.5 and 5.2 cm^{-1} for the deuterated reaction centers above and below 20 K, respectively. (●———●) KSCN-treated reaction centers, (○———○) deuterated KSCN-treated reaction centers.

Fig. 7. These results are qualitatively similar to those of the protonated reaction centers. The maximal and minimal g values are again found to lie perpendicular and parallel to the membrane plane, respectively, although with the deuterated reaction centers the total shift in the Q_I^- signal is less, approx. 9 G, which appears to arise from a smaller shift in the resonance when the magnetic field is parallel to the membrane plane.

The temperature-dependent coalescence of the Q_I^- EPR signals recorded at 0 and 90° to the magnetic field in KSCN-treated, deuterated reaction center multilayers is shown in Fig. 9. The narrower Q_I^- EPR signal in the deuterated reaction centers reveals additional asymmetry in the lineshape of this absorption. These data suggest that the signal recorded with the magnetic field parallel to the membrane plane contains two components. First, a g_y component having a temperature-dependent resonance position, which appears as a shoulder at temperature above 10 K, and moves toward g 2.005 as the temperature is raised; a second, a g_z component which remains relatively fixed near the free-electron g 2.0023 position. With the magnetic field perpendicular to the membrane plane a single g_x absorption is seen, which decreases in g value toward g 2.005 as the temperature is raised. The barely resolvable angular dependence of the broadened Q_I^- signal in protonated reaction center multilayers at temperature above 200 K can be seen to arise from the angular dependent g value shifts resolved in the deuterated samples.

The angular dependencies of the Q_I^- signals observed at temperatures abive 200 K suggest a model in which the g_y and g_z components of Q_I^- are randomly oriented in a plane parallel to the plane of the membrane surface, while the g_x direction is oriented perpendicular to the membrane surface. These results suggest an orientation in which the plane of the quinone's aromatic ring is perpendicular to the plane of the membrane surface. The origin of the anomalously large angular dependence of the Q_I^- signal at low temperature is not understood. However, the spectra from the deuterated reaction center samples suggest the possibility that either the g_x and g_y values are simultaneously shifted as the temperature is lowered, or alternatively, that the g_x and g_y values are split symmetrically about g 2.005 and show a temperature-dependent averaging or exchange.

The $LiClO_4$-treated reaction center membrane multilayers

The reconstituted $LiClO_4$-treated reaction center membrane multilayer provides an additional iron-depleted reaction center preparation in which to

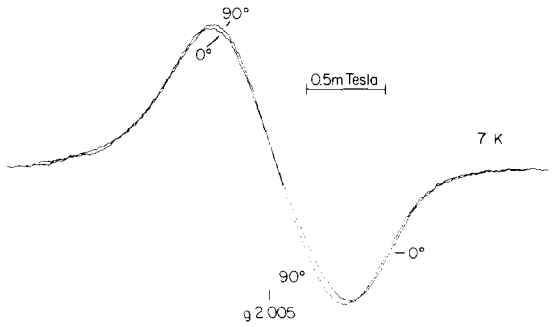


Fig. 11. Angular dependence of the Q_I^- signal in $LiClO_4$-treated, reaction center reconstituted membrane multilayers. Spectra were recorded with 1 μW of microwave power, 2.5 G modulation amplitude and gain of $6.3 \cdot 10^3$.

examine the orientation of Q_I, and is a preparation which has been designed to remove the reaction center H protein subunit [11,33].

The angular dependence of the Q_I^- EPR signal in the $LiClO_4$-treated reaction center multilayers at 7 K is shown in Fig. 11. These preparations show only a small angular dependent change in lineshape which is temperature insensitive and is analogous to that seen in the KSCN-treated samples at temperatures above 200 K.

This close similarity with the angular dependent lineshape changes in the KSCN-treated reaction center multilayers indicates that Q_I is oriented similarly in both preparations, with the g_y and g_z directions parallel and g_x direction perpendicular to the membrane plane. However, the absence of a temperture-dependent shift in EPR absorption in the $LiClO_4$-treated reaction center multilayers suggests that the unexpectly large g value shift seen at low temperature in the KSCN-trated preparations may arise from temperature-dependent changes in the association of Q_I with its protein environment, and possibly with the H subunit.

The light-induced $(BChl)_2^+$ and Q_I^- EPR signals

In the experiments described in the preceding sections, the Q_I^- species was trapped at room temperature and examined by EPR at low temperature. In order to test the possibility that significant reorientation of Q_I may occur following reduction at room temperature, we have examined the angular dependence of the light-induced $(BChl)_2^+$ and Q_I^- EPR signals generated at 8 K in the KSCN-treated reaction center membrane multilayers. The symmetric 9.7 G wide $(BChl)_2^+$ signal at 8 K is centered at g 2.0026 [9,40], and will overlap the Q_I^- signal.

Fig. 12 shows the angular dependence of the combined light-induced $(BChl)_2^+$ and Q_I^- signals in the KSCN-treated reaction center membrane multilayer. Again, the zero-crossing point of the signals is seen to shift to lower g values when the magnetic field is aligned parallel to the membrane, while the largest g values are observed with the magnetic field normal to the membrane plane. The contribution to this angular dependence due to the $(BChl)_2^+$ signal could be determined by examining the angular variation of the light-induced signal in untreated reaction center membrane multilayers. Here, the EPR absorption of the

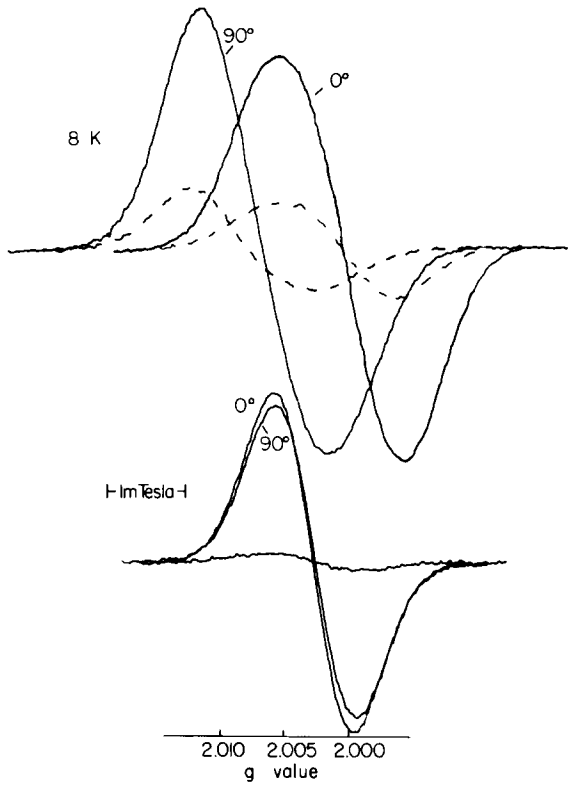


Fig. 12. Reversible light-induced EPR signals in reaction center reconstituted membrane multilayers at 8 K. Q_I was not trapped in the reduced state prior to low-temperature photochemistry (see Materials and Methods). The top spectra were generated in KSCN-treated membrane multilayers and recorded with 5 μW of microwave power, 4 G modulation amplitude, $4 \cdot 10^3$ spectrometer gain. (———) Light, (- - - - -) dark. Top, KSCN-treated reaction centers; bottom, untreated reaction centers.

companion $Q_I^- Fe^{2+}$ signal is shifted away from the g 2.002 region, and the $(BChl)_2^+$ signal can be observed uniquely. The lack of an appreciable angular dependence of the $(BChl)_2^+$ signal (Fig. 12) agrees with the previous analysis of the $(BChl)_2^+$ lineshape which predicts a minor g value anisotropy for this signal [40]. The absence of an angular dependence for the $(BChl)_2^+$ signal indicates that the observed angular dependence for the combined $(BChl)_2^+$ and Q_I^- signals arises nearly exclusively from that of the Q_I^- species.

The similarity between the shift in the resonance positions for the light-induced signals formed reversibly at 8 K and that of the Q_I^- signal trapped at

room temperature implies a similar orientation for Q_I in both cases.

Discussion

Orientation of Q_I

A perpendicular orientation of Q_I is most clearly apparent in the deuterated KSCN-treated reaction center multilayers at temperatures above 200 K, where the principal g values of Q_I^- are seen to correspond closely to those determined previously for the ubisemiquinone anion [23,24], and are seen to be oriented in the multilayer array. The g_z EPR absorption which is likely to be associated with a direction perpendicular to the quinone ring [22,39] is found to lie parallel to the plane of the membrane surface, together with one in-plane quinone direction, but the inability to resolve the overlapping g_x and g_y absorbances at these temperatures leaves uncertain the orientation of the oxygen-oxygen axis. This orientation of the $Q_I^- g$ tensor is also reflected by the small angular dependence of the much broader Q_I^- signals in the protonated KSCN-treated reaction centers at temperatures above 200 K, and in the $LiClO_4$-treated reaction center membranes at all temperatures.

The anomalous shifts in the position of the EPR absorption seen only in the KSCN-treated membrane multilayers at temperatures below 200 K lie outside of the g value anisotropy described for the isolated ubisemiquinone anion [23,24]. This suggests a possible involvement of the protein in determining the magnetic properties of Q_I in the KSCN-treated reaction centers. For example, changes in solvents and temperatures have been observed to shift the g value of semiquinones in vitro [41,42] and these have been attributed to changes in spin-orbit coupling and in the π-electron distribution arising from hydrogen and ion binding to the quinone [41,42]. Additional shifts may also be expected if the conformation or symmetry of Q_I is altered by an association within the reaction center. The coalescence of these shifts is suggestive of a possible motional averaging or resonance exchange of the g_x and g_y absorbances. The absence of these shifts in the $LiClO_4$-treated reaction center multilayers indicates that associations between the reaction center and Q_I may differ in various iron-depleted preparations, although the Q_I orientation is likely to be similar.

A direct comparison between the Q_I orientation in Fe^{2+}-depleted reaction center multilayers and that in chromatophores and Fe^{3+}-conatining, isolated reaction center preparations is being sought by a comparison of the light-induced, optical linear dichroism of Q_I in the ultraviolet. However, the close similarity in the $(BChl)_2$ triplet EPR dichroism as well as in the optical linear dichroisms of the BChl and BPh molecules (Tiede, D. and Dutton, P.L., unpublished observations) implies that significant rearrangement of the reaction center chromophores has not occurred in the iron-depleted samples.

A perpendicular orientation of Q_I with respect to the plane of the membrane surface determined here for the iron-depleted reaction center multilayers contrasts with a nearly parallel arrangement described by Hales and Gupta [22] for iron-depleted particles of *R. rubrum*. The discrepancy may arise from the differences in the bacterial species, in preparations, or in the method of analysis. The angular dependence of the Q_I^- signal in the deuterated reaction center reported here ahs provided a more direct assessment of the g tensor orientation than the lineshape analysis described by Hales and Gupta [22].

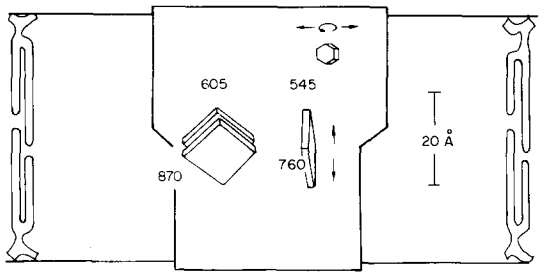
$(BChl)_2$ orientation

The orientation of the magnetic axes of the $(BChl)_2$ triplet shown here in *Rps. sphaeroides* chromatophores and reaction center preparations agrees closely with the determination made previously in chromatophore and reaction center preparations of *R. rubrum* [22]. Comparisons of the orientation of the triplet magnetic axes with the optical transitions determined by linear dichroism [19] indicate an approximate correspondence between the optical Q_y (605 nm) and Q_x (865 nm) transitions and the triplet y and x directions, respectively.

It has been pointed out [22] that in the simplest case of a dimer formed by two parallel BChl molecules, the magnetic axes of the triplet state may be analogous to their location in the monomeric BChl, i.e., having the z direction associated with a direction perpendicular to the BChl planes, and the x and y directions parallel to the BChl planes. The orientation of the $(BChl)_2$ triplet magnetic axes would then suggest a structure in which $(BChl)_2$ is nearly perpendic-

ular to the membrane, having the normal to the BChl planes tilted 10–20° away from the plane of the membrane surface.

The optical properties of $(BChl)_2$ are also consistent with the possibility of nearly aligned, parallel, stacked monomers for $(BChl)_2$, but the possibility of other structures cannot be ruled out [18–21]. If the nonorthogonal 680 nm and 870 nm $(BChl)_2$ transitions are assumed to lie approximately in the plane of the dimer, the alternative orientations of these transitions determined by linear dichroism [18–21] suggest a structure in which the plane of $(BChl)_2$ is tilted no greater than either 34 or 11° away from the membrane normal. Although more definitive experiments on the structure of $(BChl)_2$ and its interaction with other chromophores are clearly needed, the compatibility of the EPR and optical dichroisms suggests that it is worthwhile to consider a nearly perpendicular orientation of the BChl planes with respect to the membrane surface as a working model for the orientation of $(BChl)_2$ within the reaction center.



Orientation of Redox Groups in *Rps. sphaeroides* Reaction Centers

Molecular Species Determined by EPR	Angle Between Plane of Molecular Rings and Membrane Surface	Distances Between Molecular "Edges"
$(BChl)_2$ [a] BPh Q_I	70°-80° — 90°	~10 Å ≥15 Å ~10 Å
Determined by optical spectroscopy (ref. 18-21)		Angle Between Planes of Molecular Rings
$(BChl)_2$ ($\gamma_{600,870}$=75°) or $(BChl)_2$ ($\gamma_{600,870}$=105°)	± 56° ± 79°	67° 59° 89°
BPh or BPh	± 43° ± 88°	71°

[a] A 10°-20° tilt from the membrane normal has also been determined for the $(BChl)_2$ in *R. rubrum* (22)

Fig. 13. Model for the organization of the reaction center redox components. The arrows reflect uncertainties in the placement of the redox components with respect to $(BChl)_2$. The position of $(BChl)_2$ and Q_I within the profile of the membrane has been suggested from the light-induced electrochromic shifts of the carotenoids in chromatophore membranes [43,44]. Other features are discussed in the text.

Organization with the reaction center

Fig. 13 presents a working model for the molecular scale and organization of the redox groups within the reaction center. The table summarizes the angular inclinations of $(BChl)_2$ and Q_I determined in this paper, as well as the alternative arrangements of $(BChl)_2$ and the BPh intermediate determined by optical dichroism. Since the positions of the spectroscopic transitions on $(BChl)_2$ are not known, this figure assumes that the in-plane triplet EPR transitions and optical absorptions lie in plane parallel to $(BChl)_2$ as described above. In addition, the angle of intersection of the molecular planes has also been calculated from the planes defined by the optical transitions. Distances have been estimated from measurements of magnetic interaction [14–17] and electron-transfer rates [5,7,8,17]. The shape of the reaction center is that determined from X-ray [34] and neutron [42] diffraction studies. The figure presents only one of the four possible arrangements between $(BChl)_2$ and the BPh intermediate described in the optical dichroism studies [19].

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References

1 DeVault, D. and Chance, B. (1966) Biophys. J. 6, 825–847
2 Clayton, R.K. (1962) Photochem. Photobiol. 1, 201–210
3 Parson, W.W. (1967) Biochim. Biophys. Acta 131, 154–172
4 McElroy, J.D., Mauzerall, D.C. and Feher, G. (1974) Biochim. Biophys. Acta 333, 261–277
5 Peters, K., Avouris, P. and Rentzepis, P.M. (1978) Biophys. J. 23, 207–217

6 Parson, W.W., Schenck, C.C., Blankeship, R.E., Holten, D., Windsor, M.W. and Shank, C.V. (1978) in Frontiers of Biological Energetics (Dutton, P.L., Leigh, J.S. and Scarpa, A., eds.), vol. 1, pp. 37–44, Academic Press, New York
7 Hopfield, J.J. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 3640–3644
8 Jortner, J. (1976) J. Chem. Phys. 64, 4860–4867
9 Dutton, P.L., Prince, R.C. and Tiede, D.M. (1978) Photochem. Photobiol. 28, 939–949
10 Blankenship, R.E. and Parson, W.W. (1979) in Photosynthesis in Relation to Model systems (Barber, J., ed.), Elsevier/North-Holland, Amsterdam
11 Feher, G. and Okamura, M.Y. (1976) Brookhaven Symp. Biol. 28, 183–194
12 Okamura, M.Y., Isaacson, R.A. and Feher, G. (1975) Proc. Natl. Acad. Sci. 72, 3491–3495
13 Shuvalov, V.A., Klevanik, A.V., Sharkov, A.V., Matveetz, Yu.A. and Krukov, D.G. (1978) FEBS Lett. 91, 135–139
14 Tiede, D.M., Prince, R.C. and Dutton, P.L. (1976) Biochim. Biophys. Acta 449, 447–467
15 Tiede, D.M., Leigh, J.S. and Dutton, P.L. (1978) Biochim. Biophys. Acta 503, 524–544
16 Tiede, D.M., Leigh, J.S. and Dutton, P.L. (1978) in Frontiers of Biological Energetics (Dutton, P.L., Leigh, J.S. and Scarpa, A., eds.), vol. 1, pp. 45–53, Academic Press, New York
17 Okamura, M.Y., Isaacson, R.A. and Feher, G. (1979) Biochim. Biophys. Acta 546, 394–417
18 Vermeglio, A. and Clayton, R.K. (1976) Biochim. Biophys. Acta 449, 500–515
19 Rafferty, C.N. and Clayton, R.K. (1979) Biochim. Biophys. Acta 546, 189–206
20 Clayton, R.K., Rafferty, C.N. and Vermeglio, A. (1979) Biochim. Biophys. Acta 545, 58–68
21 Vermeglio, A., Breton, J., Paillotin, G. and Gogdell, R. (1978) Biochim. Biophys. Acta 501, 514–530
22 Hales, B.J. and Gupta, A.D. (1979) Biochim. Biophys. Acta 548, 276–286
23 Feher, G., Okamura, M.Y. and McElroy, J.D. (1972) Biochim. Biophys. Acta 267, 222–226
24 Loach, P.A., Kung, M. and Hales, B.J. (1975) Ann. N.Y. Acad. Sci. 244, 297–319
25 Dutton, P.L., Petty, K.M., Bonner, H.S. and Morse, S.D. (1975) Biochim. Biophys. Acta 387, 536–556
26 Clayton, R.K. and Wang, R.T. (1971) Methods Enzymol. 23, 696–704
27 Clayton, R.K. and Yau, H.F. (1972) Biophys. J. 12, 867–881
28 Blankenship. R.E. and Parson, W.W. (1979) Biochim. Biophys. Acta 545, 429–444
29 Packham, N.K., Tiede, D.M., Mueller, P. and Dutton, P.L. (1980) Proc. Natl. Acad. Sci. U.S.A., 77, 6339–6343
30 Wraight, C.A. (1977) Biochim. Biophys. Acta 459, 525–531
31 Vermeglio, A. (1977) Biochim. Biophys. Acta 459,
32 Okamura, M.Y., Steiner, L.A. and Feher, G. (1974) Biochemistry 13, 1394–1403
33 Okamura, M.Y., Debus, R.J., Isaacson, R.A. and Feher, G. (1980) Fed. Proc. 39, 1802
34 Pachence, J.M., Dutton, P.L. and Blasie, J.K. (1979) Biochim. Biophys. Acta 548, 348–373
35 Singleton, W.S., Gray, M.S., Brown, M.L. and White, J.L. (1965) J. Am. Oil Chem. Soc. 42, 53–63
36 Leigh, J.S. and Dutton, P.L. (1974) Biochim. Biophys. Acta 357, 67–77
37 Thurnauer, M.C., Katz, J.J. and Norris, J.R. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 3270–3274
38 Romijn, J.C. and Amesz, J. (1977) Biochim. Biophys. Acta 461, 327–338
39 Hales, B.J. (1975) J. Am. Chem. Soc. 97, 5992–5997
40 McElroy, J.D., Feher, G. and Mauzerall, D.C. (1972) Biochim. Biophys. Acta 267, 363–374
41 Zandstra, P.J. (1964) J. Chem. Phys. 41, 3655–3656
42 Pachence, J.M., Dutton, P.L. and Blasie, J.K. (1981) Biochim. Biophys. Acta 635, 267–283
43 Dutton, P.L., Petty, K.M., Bonner, H.S. and Morse, S.D. (1975) Biochim. Biophys. Acta 387, 536–556
44 Jackson, J.B. and Dutton, P.L. (1973) Biochim. Biophys. Acta 325, 102–113