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Electric-field dependence of the quantum yield in reaction centers of photosynthetic bacteria

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Multilayer Langmuir–Blodgett films of reaction centers from the photosynthetic bacterium *Rhodopseudomonas sphaeroides* have been fabricated with partial net orientation. The films showed substantial electrical response under pulsed illumination. From measurements of the light-induced voltage generated across the Langmuir–Blodgett film, we have succeeded in quantitating the electric-field dependence of the quantum yield of charge separation in photosynthesis. The results presented here are compared with our previous determination of the field effect on quantum yield, in which flash-activated charge separation as a function of the applied field was assayed by the extent of bacteriochlorophyll dimer, $(BChl)_2$, oxidation measured optically at 860 nm. The two methods provided consistent dependencies of quantum yield on applied electric field. Analysis of the data reveals that the quantum yield of $(BChl)_2^+BPhQ_A^-$ formation decreases from a value of 0.96 at zero applied field to about 0.75 for a field of 120 mV/nm vectorially directed to hinder light-activated electron transfer. For oppositely applied fields, the quantum yield saturates at unity. The source of the effects is considered to reside in the electric field dependence of the free-energy difference between the energy levels that are involved in the initial charge separation between the $(BChl)_2$ in the first singlet excited state, $(BChl)_2^*$, through the bacteriopheophytin, BPh, to the primary ubiquinone, Q_A. Possible contributions to the field-induced loss of quantum yield of $(BChl)_2^+BPhQ_A^-$ formation are: (1) a decrease in the free-energy gap between the states $(BChl)_2^*$ and $(BChl)_2^+BPh^-Q_A$, leading to an increased rate of decay via the excited singlet state back to the ground state; (2) a stimulated return from $(BChl)_2^+BPh^-Q_A$ directly or via the $(BChl)_2$ triplet state to the ground state and (3) an impeded electron transfer from $(BChl)_2^+BPh^-Q_A$ to $(BChl)_2^+BPhQ_A^-$. These possibilities are discussed. Correlation of the electrical response with measurements of the photo-induced absorbance change allows determination of the projection of the electron-transfer distance on the normal to the plane of the film, which is in good agreement with previous measurements using different techniques.

Introduction

A feature common to photosynthetic reaction centers is light-induced electron transfer [1]. In the reaction centers of the photosynthetic bacterium *Rhodopseudomonas sphaeroides*, this process in-

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Abbreviations: LDAO, lauryldimethylamine *N*-oxide; RC, reaction center; BPh, bacteriopheophytin; BChl, bacteriochlorophyll.

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volves several redox components contained within the protein. Following light absorption a bacteriochlorophyll dimer, (BChl)$_2$, assumes an excited singlet state, (BChl)$_2^*$, and transfers an electron, possibly via a monomeric bacteriochlorophyll (BChl) to a bacteriopheophytin (BPh) to form (BChl)$_2^+$BPh$^-$. Before useless recombination can occur, the BPh$^-$ reduces a ubiquinone-10 molecule, designated Q$_A$, to form (BChl)$_2^+$BPhQ$_A^-$. The rate constants, energy levels and distances between the different components in the reaction center are summarized in Fig. 1. (The values for the forward rates are reported in Refs. 2–6. The recombination rates are reported in Refs. 5 and 7–9. The value for ΔG between (BChl)$_2^*$BPhQ$_A$ and (BChl)$_2^+$BPh$^-$Q$_A$ is reported in Refs. 11 and 12; the value for ΔG between (BChl)$_2^+$BPh$^-$Q$_A$ and (BChl)$_2^+$BPhQ$_A^-$ is given in refs. 13–15. The scaling distance of 4.4 nm between the non-heme iron and the cytochrome *c* iron was obtained by resonance X-ray diffraction [16]). In the native membrane the Q$_A$ reduces a second ubiquinone (Q$_B$) in 100 μs half-time and a cytochrome *c* reduces the (BChl)$_2^+$ in microseconds to stabilize the process further [1,17]. The reaction center is considered to span the cytoplasmic membrane [18–20] with cytochrome *c* and Q$_B$ associated with the reaction center, but located on opposite sides of the membrane. Thus, the system is organized so that following light excitation electron transfer is coupled to the separation of charges within the protein directed across the membrane.

Expressions of the charge separation in photosynthetic bacteria have been seen in vivo as electrochromic responses of the carotenoid complement of the membrane [21,22], by enhanced fluorescence yield indicative of a reversal of the light reaction [23] and by shifts in redox equilibria between cytochrome *c* and (BChl)$_2$ [24]. Direct measurements of the charge separation have since been made in vitro with reaction centers incorporated into planar phospholipid bilayer membranes [25–30], as monolayers on solid supports [31] and on the interfacial region of immiscible liquids [32]. See also Ref. 33 for a review.

There is a consensus of agreement from the different approaches that the separation of charge across the membrane is effected by several distinct contributing electron-transfer steps. Electron transfers from ferrocytochrome *c* to (BChl)$_2^+$ contributes in the region of 40–50% of the separation of charge across the membrane [22,24,27,29], while electron transfer from Q$_A^-$ to Q$_B$ contributes little or nothing to the transmembrane charge separation [22,26,27,29]. Thus the charge separation accounting for the remaining 50–60% of the membrane is provided by electron transfer from (BChl)$_2^*$ to Q$_A$. The electrical response from (BChl)$_2^*$ to BPh is two-fold greater than from BPh$^-$ to Q$_A$ [32]; this indicates a 35–40% span contributed by electron transfer from (BChl)$_2^*$ to BPh and a 15–20% contribution by the BPh$^-$ to Q$_A$ reaction. Independent studies have suggested a similar (i.e., approx. 1/7 of the membrane thickness) value for the BPh to Q$_A$ span [28–30].

The work reported here focuses on the light-activated electrogenic reactions that occur between the (BChl)$_2$ and Q$_A$. The goal is to alter the reactions significantly by applying electric fields across planar arrays of reaction centers to obtain information on the energetics and factors that govern kinetics of electron transfer. Application of electric fields along the pathway of charge separation will change the relative energy gaps between each reaction step. However, since (a) each of the individual reactions between (BChl)$_2$ and Q$_A$ contributes only a small fraction of the total distance of charge separation within the protein, and (b) there is a large free-energy drop, perhaps as much as 0.9 eV, from (BChl)$_2^*$ to Q$_A$ [1,17] it is anticipated that substantial electric fields may be required to effect measurable changes in the quantum yield of (BChl)$_2^+$BPhQ$_A^-$ formation.

An experimental vehicle to achieving our goal is provided by reaction centers as monolayer films deposited by Langmuir–Blodgett techniques on conductive substrates [31]. When coated with a blocking polymer polymer layer, such films, in contrast to the systems based on planar bilayers separating aqueous phases, have been shown to withstand externally applied electric fields of up to 200 mV/nm [34]. With the capability to apply such high fields to samples with asymmetrically oriented 'up' and 'down' reaction center populations we have been able to determine quantitatively the electric field dependence of quantum yield of formation of (BChl)$_2^+$BPhQ$_A^-$ by direct electrical measurements. If one assumes that at high fields of proper orientation the quantum yield saturates at unity, the resulting zero-field yield is in good agreement with previous measurements [35–37].

Materials and Methods

Techniques

Reaction center preparation. The reaction centers were isolated from the photosynthetic bacterium *Rhodopseudomonas sphaeroides* (R26) using the method developed by Clayton and Wang [38] and modified by Okamura et al. [39]. The preparations used in this work contained variable percentages of secondary quinone, Q_B, ranging from 30% to 80% as estimated by the percent of slow phase charge recombination [40]. Nevertheless, it was observed that in the Langmuir–Blodgett films used in these measurements all of the reaction centers became equivalent with only a small percent of Q_B activity [41]. Furthermore, since Q_A-to-Q_B electron transfer does not contribute to the generation of electric potential across the membrane [27,28], the electrical measurements reported here are independent of the Q_B content of the preparation.

Electrical cell construction. The techniques used for the preparation of the quartz substrates, involving deposition of the conductive and blocking layers [42] in the sandwich cell (see Fig. 2), were the same as described in Ref. 34. Indium-tin-oxide is sputtered onto a quartz slide followed by a sputtered SiO_2 blocking layer. The reaction centers are deposited as several monolayers on this surface from a Langmuir–Blodgett film balance. The cell is dip-coated with a polymer layer (hydrogenated polyolefin) out of hexane solution. After drying, an indium layer is vacuum evaporated to complete the cell. Prior to spreading of the reaction center film on the air-water interface of the Langmuir–Blodgett trough, 50 μl 0.1% lauryldimethylamine *N*-oxide (LDAO) appropriately buffered to pH 8.0 with 10 mM Tris-HCl, was spread on the water surface. The initial area for spreading was adjusted so that the LDAO increased the surface pressure to 10 mN/m. This preparatory step was found to improve the photochemical activity and the electrical response of the reaction center films [34]. The reaction centers were picked up from the trough as the coated quartz slide was lifted from the subphase through the surface and into the air. In this film the side of reaction center that was disposed toward the aqueous phase of the trough adhered to the SiO_2 blocking layer of the slide.

Electrical and optical measurements. Photo-induced electrical transients were measured using a modified RC circuit in which the noise associated with the power supply was eliminated by using a 1 μF capacitor and a set of relays [43]. In the signal averaging measurements possible charge accumulation in the sample was avoided by applying the following biasing sequence: positive voltage, zero voltage, negative voltage, zero voltage etc. As input amplifier, a Tektronix oscilloscope model 7633 was used equipped with the 7A22 differential amplifier plug in unit. Excitation light pulses were obtained from an electronic flash (Metz model Mecablitz 45 CT-1); the pulse duration used was 50 μs. The applied sample bias was turned on about 2 ms prior to the excitation light pulse and maintained for about 10 ms, the duration of the transient measurements. Light from the flash excited the sample at near normal incidence; the light intensity was adjusted to give 5% of the maximum observable voltage to insure a linear response. Data were recorded using a Cromemco System Three microcomputer, which also controlled the timing of events in the measurement sequence. The electric field applied to the sample was determined form the known sample cell area, A (approx. 45 mm^2), capacitance, C (approx. 900 pF); applied voltage, V (from -150 to $+150$ $mV \cdot nm^{-1}$), and dielectric constant of the reaction centers proteins ($\epsilon_r = 3$) [44,45] by using the equation

$$E = \frac{CV}{\epsilon_0 \epsilon_r A} \tag{1}$$

A probe beam of 860 nm light was used in the second part of our experiment to determine the degree of sample bleaching after a saturating flash. The probe beam was incident at 45° onto the quartz slide side of the sample and was reflected back from the top metal electrode, thus making a double pass through the reaction center layer. In bleaching and action spectra measurements monochromatic illumination was obtained by using an Instruments S.A. Inc. H-20 monochromator with 1 mm slits.

Theory

Effect of an external field on the energy levels. The attainment of the charge separated state $(\mathrm{BChl})_2^+\mathrm{BPhQ}_A^-$ involves the formation of at least two dipoles; d_1, associated with $(\mathrm{BChl})_2^+\mathrm{BPh}^-\mathrm{Q}_A$ and d_2, associated with $(\mathrm{BChl})_2^+\mathrm{BPhQ}_A^-$, whose values are given by:

$$d_1 = ex_1 \tag{2}$$

$$d_2 = ex_2 \tag{3}$$

where x_1 is the vector between BPh and $(\mathrm{BChl})_2$, and x_2 that between Q_A and $(\mathrm{BChl})_2$, and e is the elemental charge. In the presence of an external field, the energy of a dipole, U, is given by:

$$U = -\mathrm{d}E \tag{4}$$

If one assumes that (a) the external field is homogeneous and equal to the induced local field and (b) that the projected distances between the chromophores along the field axis are as given in Fig. 1b, the positions of the energy levels relative to the ground and excited singlet states are presented in Fig. 1c as a function of the field over the range used in our experiments.

Quantum yield as a function of external fields. Let us assume that a Langmuir–Blodgett film of reaction centers with an asymmetric up and down population is incorporated into a sandwich cell as seen in Fig. 2. Call the up population P_1 and the down population P_2. We can define the parameter, δ, describing the degree of asymmetry, as the fraction of unpaired reaction centers:

$$\delta = \frac{P_1}{P_1 + P_2} - \frac{P_2}{P_1 + P_2} \tag{5}$$

The fraction of reaction centers with up and down orientations will then be given by $(1+\delta)/2$ and $(1-\delta)/2$, respectively. For a completely unoriented film $\delta = 0$, while for a fully oriented film $\delta = \pm 1$.

A light-induced voltage is observed in the cell as a result of charge separation in the reaction centers. Its absolute value depends on the degree of asymmetry and its sign will depend on the direction in which the larger of the two populations is oriented. Let us define the relative photoresponse:

$$R(E) = \frac{\Delta V(E)}{I} \tag{6}$$

where I is the intensity of the pulsed illumination.

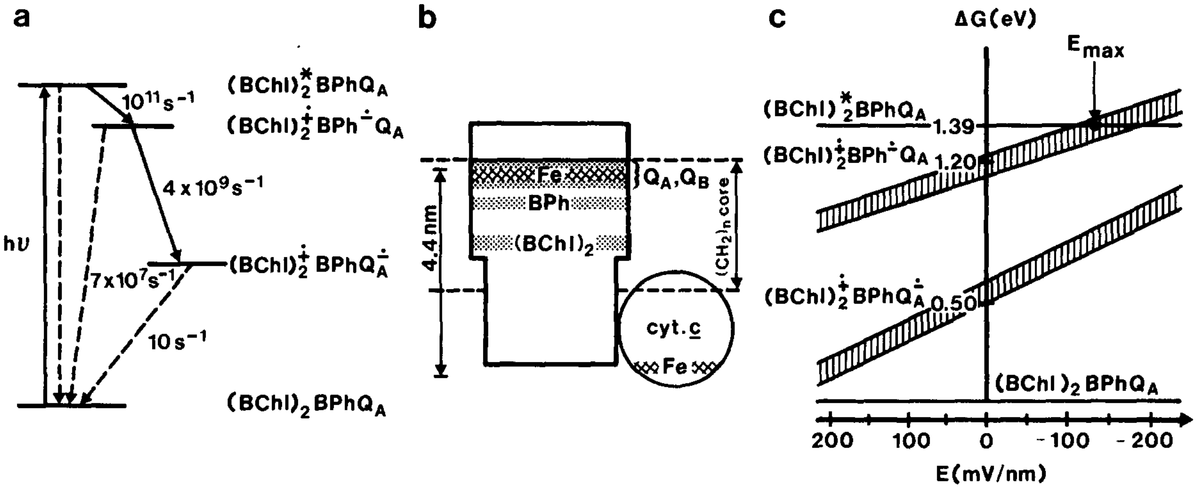

Fig. 1. (a) Electron-transfer rates in reaction centers of *Rps. sphaeroides*. (b) Schematic representation of the 'dielectric' distances between $(\mathrm{BChl})_2$, BPh, and Q_A, Q_B. The shaded areas indicate the uncertainties in these distances. Cyt *c*, cytochrome *c*. (c) Electric field dependence of the energy levels of the states $(\mathrm{BChl})_2^+\mathrm{BPh}^-\mathrm{Q}_A$ and $(\mathrm{BChl})_2^+\mathrm{BPhQ}_A^-$, according to Eqn. 4 with distances between chromophores (as given in Fig. 1b) being 1.2 nm between $(\mathrm{BChl})_2$ and BPh and 0.6 nm between Q_A and BPh. For both states the shaded areas represent the range of field dependencies corresponding to the uncertainties in the zero field energy levels.

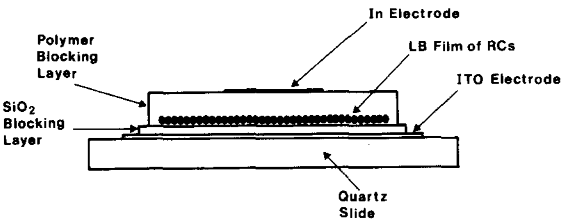

Fig. 2. Schematic representation of the sample cell used in the measurements ITO, indium-tin-oxide; LB, Langmuir–Blodgett.

For low light intensities the photo-induced voltage change, ΔV, will be proportional to the light intensity, and the relative photoresponse, R, will be light-intensity independent.

We now introduce the field-dependent quantum yield for charge separation $\phi(E)$ which is a measure of the fraction of reaction centers that reach the state $(BChl)_2^+Q_A^-$ after excitation. In the time scale of our measurements the amount of charge separation generated at a given field by the population P_1 will be proportional to $\phi(E)P_1$, while that of population P_2 will be proportional to $\phi(-E)P_2$. For low light intensities the observed voltage drop will be given by:

$$\Delta V(E) = \frac{\phi(E)P_1 - \phi(-E)P_2}{c} I \tag{7}$$

where c is a constant dependent on the cell geometry, capacitance and dielectric constants of the system. The relative photoresponses for positive and negative applied fields can then be written as:

$$R(E) = \alpha[(1+\delta)\phi(E) - (1-\delta)\phi(-E)] \tag{8}$$

$$R(-E) = \alpha[(1+\delta)\phi(-E) - (1-\delta)\phi(E)] \tag{9}$$

where $\alpha = (P_1 + P_2)/2c$.

In order to facilitate our subsequent analysis of the experimental data, we now introduce two new quantities $R_{av}(E)$ and $R_{diff}(E)$, defined by:

$$R_{av}(E) = \frac{R(E) + R(-E)}{2} = \delta\alpha[\phi(E) + \phi(-E)] \tag{10}$$

$$R_{diff}(E) = \frac{R(E) - R(-E)}{2} = \alpha[\phi(E) - \phi(-E)] \tag{11}$$

which are respectively even and odd functions of the electric field. Eqns. 10 and 11 give the following dependencies of the yield on the electric field,

$$\phi(E) = \frac{1}{2\alpha}\left[\frac{R_{av}(E)}{\delta} + R_{diff}(E)\right] \tag{12}$$

$$\phi(-E) = \frac{1}{2\alpha}\left[\frac{R_{av}(E)}{\delta} - R_{diff}(E)\right] \tag{13}$$

The method of determination of parameters α and δ will be dealt with in the next section.

Action spectrum of light-induced voltage in the reaction center Langmuir–Blodgett films

In order to verify that the observed light-induced electrical signal is associated with the charge displacement in the reaction center, the electrical action spectrum of the sample cell was recorded without an applied electric field bias. In Fig. 3 the action spectrum is compared with the absorption spectrum of the same batch of reaction centers in aqueous-detergent solution. The structure of the two spectra demonstrates that the observed photovoltages are the result of light absorbed by the reaction centers. There are differences, however, in certain regions both in amplitudes and in peak

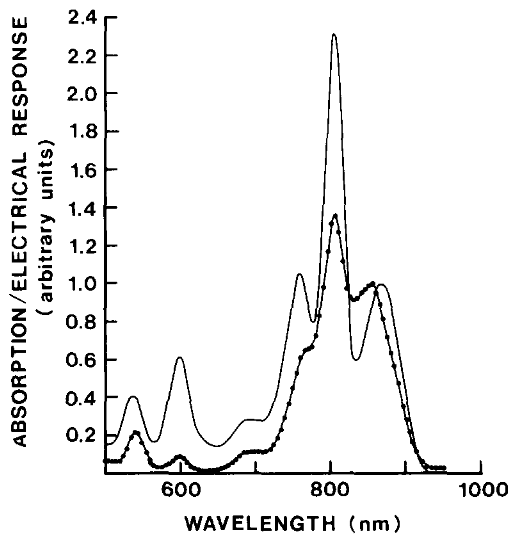

Fig. 3. Absorption spectrum of reaction centers in solution (full line), and photoinduced electrical action spectrum (at zero applied bias) of reaction centers from the same batch in a Langmuir–Blodgett film (connected discrete points). Varying throughput of the optical system and efficiency of the detector over the wavelength range investigated have been considered and the action spectrum properly normalized.

positions. These differences could arise from several sources. For example, effects from the degree of hydration of the film and the presence of detergent and contributions from polarization, resulting from the geometry of the optical cell and other optical effects, cannot be ruled out. It is also conceivable that energy transfer from certain chromophores to the $(BChl)_2$ is less efficient than that encountered in solution. These points deserve further study, but important to the present work is the clear demonstration that the photovoltages are generated by light absorbed by the chromophores of the reaction center.

Comment on the relationship between hydration and activity of reaction center films

Clayton [46] working on dried films of chromatophores and reaction centers of photosynthetic bacteria revealed the important role of the hydration state of the reaction center both in the quantum yield of charge separation and in the kinetics of the charge recombination process. He reported that a high degree of dehydration resulted in blue shifts in all the absorption peaks of the reaction center as well as a distortion of the 860 nm band associated with $(BChl)_2$. Multiphasic charge recombination kinetics and quantum efficiency drops of up to 60% were observed. All these effects were reported to be reversible upon rehydration.

In a more detailed study of the effect of hydration on the photosynthetic process, Nikolaev et al. [47] concluded that some of the effects reported by Clayton appeared only after the relative humidity of the films was down to 70% to 90% below saturation and they interpreted this as the point at which no relatively free water is left.

In the work reported here, no efforts were made to control or measure the ambient humidity. However, spectrophotometric assays of the Langmuir–Blodgett films (before evaporation of the second electrode) were routinely performed. It was found that while spectra of the reaction center films did indicate some minor blue shifts in the absorption bands, they were essentially the same as those presented by Tiede et al. [31]; the dramatic distortion of the 860 nm band evident in specifically dried films and commensurate with loss in quantum yield was not observed. The amplitude of $(BChl)_2^+$ generated and the charge recombination in the electron transfer from Q_A^- back to $(BChl)_2^+$ [34,41] were the same whether examined in the films submerged in water or in air before and after deposition of the second electrode. Thus we have concluded that, while humidity below a certain critical value can have major deleterious effects on reaction center function, the work presented here was performed well above this level. The loss of activity that is encountered in reaction center films (approx. 50%) is not related to the degree of hydration; this loss results from damage, exact source(s) currently unknown, incurred during manipulation of the protein on the Langmuir–Blodgett trough.

Results and Discussion

Fig. 4 shows the field dependence of the relative photoresponse $R(\pm E)$ for two different reaction center Langmuir–Blodgett films labelled A and B. The measured photo-voltages were typically up to a few mV in the regime where photovoltage was proportional to the light intensity. It was observed that the photo-voltage at zero applied field after either positive or negative bias remained constant throughout the experiment, showing that the reaction center films were not damaged by repetitive cycles of illumination and electric field application.

As observed previously [31,41], under zero field conditions, the photo-induced voltage yielded a negative indium-tin-oxide electrode relative to the Al electrode (see Fig. 2). Since under zero-field conditions the unpaired fraction of reaction centers, δ, entirely determines the sign of the electrical response, from the sign of the observed response we conclude that the quinone-containing end of the reaction center protein is preferentially oriented toward the indium-tin-oxide electrode. In turn we can conclude that in the film at the air-water interface of the Langmuir–Blodgett trough a predominating population of the reaction centers are oriented with their Q_A containing side toward the water subphase. The convention used in the present measurements is that δ is positive; therefore of the two vectorially up and down populations P_1 and P_2, $P_1 > P_2$, and P_1 is the population with the Q_A side disposed toward the

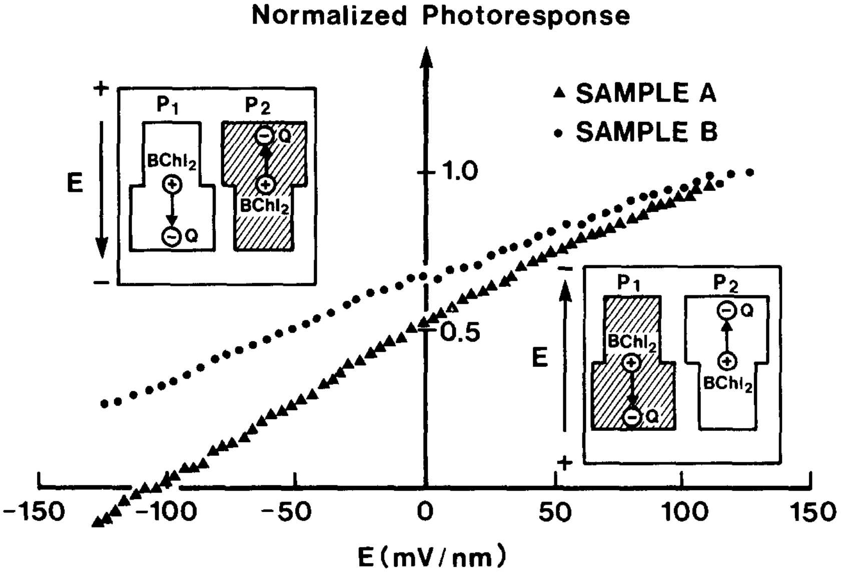

Fig. 4. Unity-normalized photoresponse as a function of the applied field, E; ▲, sample A, ●, sample B. Populations P_1 (quinone side toward indium-tin-oxide) and P_2 have been schematically illustrated in the insets. The hatching indicates the population whose charge separation is assisted by the external field.

water phase and hence the indium-tin-oxide electrode. The response under zero field conditions (i.e., indium-tin-oxide negative; Al, positive) corresponds by convention to a positive relative photoresponse ($R(0)$, Eqn. 6). Similarly, fields that assist the charge separation in the unpaired P_1 population are regarded as positive, i.e., the applied field makes the indium-tin-oxide electrode positive relative to the Al electrode. In general, as shown in Fig. 1c, fields that assist charge separation are considered to do so by increasing the free-energy difference between the (BChl)$_2^*$BPhQ$_A$ and (BChl)$_2^+$BPhQ$_A^-$ levels, while fields that hinder this charge separation decrease this free energy gap.

Under the influence of positive fields the P_1 population is expected to saturate in photoresponse at high field values as the quantum yield approaches unity. In contrast, under these conditions, the P_2 population will be subject to a hindering effect of the field and hence the number of reaction centers in the P_2 population reaching the charge separated (BChl)$_2^+$BPhQ$_A^-$ state will decrease. As a result, the relative photoresponse $R(E)$ (Eqn. 6) increases as seen in Fig. 4.

The application of a negative field does just the opposite; as the negative field is increased fewer reaction centers in the population P_1 attain the state (BChl)$_2^+$BPhQ$_A^-$, while the contribution from P_2 increases and as result $R(E)$ becomes smaller (Fig. 4). For a high enough negative field the contributions from both populations are identical in magnitude but have opposite signs resulting in zero overall relative photoresponse. For sample A this is indeed observed in the range of fields studied: it shows $R(E) = 0$ at $E \approx -110$ mV/nm. From Eqn. 9 it follows that the condition for zero relative photoresponse can be expressed as:

$$(1+\delta)\phi(-E) = (1-\delta)\phi(+E). \tag{14}$$

This shows that for a symmetric sample ($\delta = 0$) zero photoresponse is obtained at $E = 0$, an intuitively obvious result. If $\phi(E)$ is a monotonic function of E, this equation also predicts that zero relative photoresponse is reached at increasingly higher field as the degree of asymmetry, δ, increases.

In order to determine $\phi(E)$ we must know the different quantities appearing in Eqns. 12 and 13. $R_{av}(E)$ and $R_{diff}(E)$, shown in Fig. 5, are obtained directly from measurements of $R(E)$ and

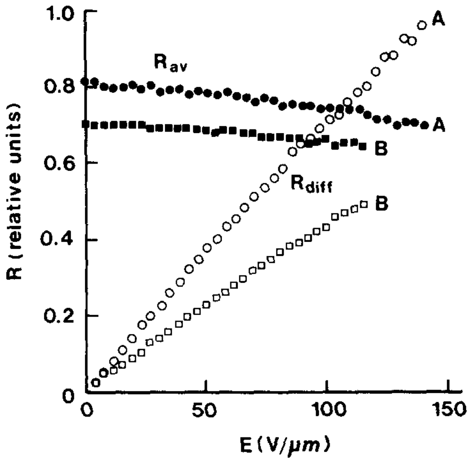

Fig. 5. Electric field dependence of photoresponses, R_{av} and R_{diff}, defined by Eqs. 10 and 11, for two different samples denoted A and B.

$R(-E)$, by using the same magnitude but opposite polarity of the bias applied to the sample. In general, direct determination of the parameters α and β is a non-trivial task involving some uncertainties. Fortunately, photosynthesis is a very efficient process with the quantum yield of charge separation approaching unity [35–37]. The yield is therefore expected to saturate for large positive fields. By stipulating the condition $\phi(E)=1$ for any two large enough positive (assisting) fields it would in principle be possible to obtain α and δ from Eqn. 12 by use of the corresponding $R_{av}(E)$ and $R_{diff}(E)$ values. However, due to inherent statistical variations, we have chosen to determine α and δ by least-squares minimization of the deviation of the experimental curve defined by Eqn. 12, from the specified unity value over an appropriate voltage range.

The unknown parameters α and δ in Eqn. 12 and 13 were determined by stipulating the condition $\phi(E)=1$ for 75 mV/nm $< E <$ 140 mV/nm and minimizing the function

$$F(\alpha,\delta)=\sum_i\left[\frac{R_{av}(E_i)}{2\alpha\delta}+\frac{R_{diff}(E_i)}{2\alpha}-1\right]^2 \qquad (15)$$

where the summation is over the experimentally employed field values which lie in the specified range. Higher field values were omitted from the least-square fit to determine α and δ as they show increased noise, probably due to the onset of electrical breakdown in the sample. The determined values of δ for the reaction center films labelled A and B were 0.134 and 0.170, which correspond to up and down populations of reaction centers of 57%–43% and 59%–41% for A and B, respectively.

The forgoing considerations permit us to present what can be regarded as the field effect on the quantum yield (ϕ) in a reaction center population where all reaction centers are oriented in the same direction (Eqns. 12 and 13). This result is shown in Fig. 6. At high positive fields ϕ is seen to saturate as expected. The saturation value in our monolayers has been assumed to be unity. With the introduction of this scaling factor we find that as the field is diminished to zero ϕ assumes the value of 0.96. This is in good agreement with previous measurements of absolute quantum yields for reaction centers in solution in the absence of external fields [35–37] as well as for air-dried films of reaction centers [46]. As the field becomes negative, hindering the charge separation process, the quantum yield decreases in a hyperbolic manner and reaches a value of approx. 0.75 at the field of −120 mV/nm.

The results reported here can be compared with our previous studies [34] in which the charge separation was determined spectrophotometrically by measuring the light induced bleaching at 860 nm, indicative of $(BChl)_2^+$ formation. The influence of the electric field on this quantity, that is the

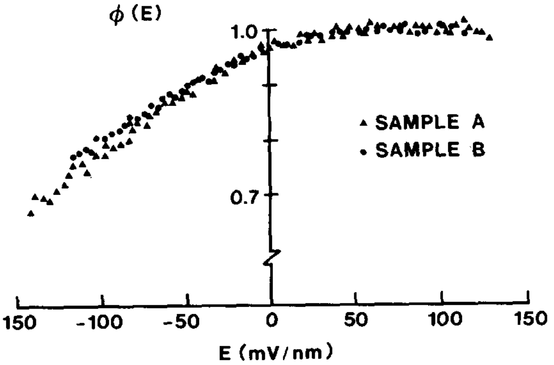

Fig. 6. Dependence of the quantum yield of charge separation, ϕ, on the electric field, E, deduced from data presented in Fig. 5.

degree of attenuation of the bleaching, was called the quenching of bleaching, $Q_b(E)$, and was defined as:

$$Q_b(E) = 1 - \frac{\phi(E) + \phi(-E)}{2\phi(0)} \tag{16}$$

which, when combined with Eqns. 12 and 13 becomes:

$$Q_b(E) = 1 - \frac{R_{av}(E)}{R_{av}(0)} \tag{17}$$

In contrast to the present work, the previous measurements gave only an average of the field dependence of the yield over the up- and down-field directions. The shape of the $\phi(E)$ curve was therefore not known nor was the correlation between the sign of the change in ϕ and the field direction. It is nonetheless possible to demonstrate the equivalence of the two different experiments. This is done by calculating, via Eqn. 17, the values of $Q_b(E)$ from the $R_{av}(E)$ values determined by electrical measurements, and by comparing them with the optically measured $Q_b(E)$ values Fig. 7 shows that there is a very good agreement between both sets of data.

The field dependence of the quantum yield is governed by the field dependences of different rate constants involved in the charge separation and recombination processes, as shown in Fig. 1a. However, from the experiment reported here it is not possible to establish which rates are responsible for the drop in yield. Indeed, as far as we are aware neither is there information available in the published literature that will contribute to providing answers to this question. Information from Fig. 1b and c does allows us to predict expected free energy changes as a function of the applied electric field. The knowledge of free-energy changes determines only the ratio of the rates involved in equilibrium, but cannot resolve the dependences of the individual rates.

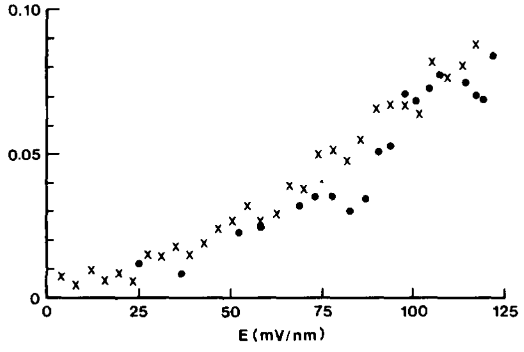

Fig. 7. Equivalence between optical and electrical determinations of the electric field influence on quantum yield of formation of $(BChl)_2^+BPhQ_A^-$. ●, quenching of bleaching as a function of field [34]. ×, quenching of bleaching calculated using Eqn. 17 with $R_{av}(E)$ determined by electrical measurements (Fig. 5). (The data from [34] have been rescaled somewhat for a comparison, since a dielectric constant of 2.6 (mistakenly reported as 3.0 in Ref. [34]) was used there for calculating the electric field.)

Despite the paucity of kinetic information, some points can be made. It is clear from Fig. 1c that the largest relative energy perturbation introduced by external field is associated with the first step, the $(BChl)_2^*BPhQ_A$ to $(BChl)_2^+BPh^-Q_A$ transition; at a field of less than 150 mV/nm the energy gap is reduced from 180 mV to zero. Over the same range the energy difference between $(BChl)_2^+$-BPh^-Q_A and $(BChl)_2^+BPhQ_A^-$ has diminished by only about 15% of the zero-field value, and the energy gap between $(BChl)_2^+BPh^-Q_A$ and the ground state has increased by only 15%. Finally the energy gap for recombination from $(BChl)_2^+$ $BPhQ_A^-$ to the ground state increases by approx. 50%. From these considerations it appears that the most probable major source of quantum yield drop lies in the first step, the transition(s) involved in converting $(BChl)_2^*BPhQ_A$ to $(BChl)_2^+BPh^-Q_A$. Some evidence against the $(BChl)_2^+BPh^-Q_A$-to-$(BChl)_2^+BPhQ_A^-$ reaction rate being sufficiently sensitive to the energy gap to account for the drop in quantum yield is provided by experiments in which this energy gap was diminished by replacement of the native Q_A (ubiquinone) with other lower redox potential quinones [48,49]; in this work the reaction rate was slowed by only 2–3-fold when the energy gap was diminished by 15% (if this reaction was solely responsible for the observed loss in quantum yield a greater than 50-fold slowing would be required). Although not of primary importance to the value of the quantum yield the $(BChl)_2^+BPhQ_A^-$ to ground state reaction has also been shown to be insensitive to the energy gap; again, a 50% reduction by either application of electric fields [41] or quinone replacement [49] has only a 5-fold slowing effect. The dependence of the rate of decay of $(BChl)_2^+BPh^-Q_A$ to the ground state either directly or via the $(BChl)_2$ triplet state is, however, of primary importance to the assignment of the sources of loss in quantum yield. We have at present no information on this important matter. However, experiments addressing the yield of formation and time-resolved decay of the $(BChl)_2^+BPh^-Q_A$ state as a function of electric field are currently at the preliminary stages. The question of what are the routes for decay of the $(BChl)_2^+BPh^-Q_A$ state and how are they affected by electric fields has been addressed before by others. Work with *R. rubrum* chromatophores by Van der Wal et al. [50] and Godik [10] on the effect of membrane potential on delayed fluorescence yield and lifetime has shown that the major electric-field sensitive reaction was in the initial steps between $(BChl)_2^*BPh$ and $(BChl)_2^+BPh^-$; other routes were considered insensitive to the electric fields. Thus the working model is that the field sensitive reactions that govern quantum yield are located in the stages of photosynthetic charge separation before and including the formation of $(BChl)_2^+BPh^-Q_A$.

Some final comments can be made on the vectorial asymmetry of the reaction center molecules in the films and the distance estimates between the reactants. Once the asymmetry parameter, δ, is known, the projected electron displacement, Δx normal to the plane of the Langmuir–Blodgett film, can be determined, provided the number of active reaction centers per unit area of the film, N_{RC}, and the maximum photo-induced voltage at zero field, $\Delta V_{max}(0)$, in the sample are also known. The last two quantities can be obtained experimentally. $\Delta V_{max}(0)$ is measured directly, while N_{RC} is given by:

$$N_{RC} = \frac{N}{1000}\,\frac{\cos\theta}{2\Delta\epsilon_{860} f}\,{}^{10}\log\left(1 + \frac{\Delta I_{probe}}{I_{probe}}\right) \tag{18}$$

where θ is the angle of incidence of the probe beam onto the slide, $\Delta\epsilon_{860} = 11.2\ mM^{-1} \cdot mm^{-1}$ is the change of extinction coefficient at 860 nm when the reaction centers are fully bleached [51], f is a correction factor applied to the extinction to allow for probe beam polarization and non-random orientation of the 860 nm transition moment of $(BChl)_2$) in the oriented reaction centers in the films, and the fraction in the argument of the logarithm represents the maximum change of the 860 nm probe beam intensity due to sample bleaching at zero field. Eqn. 21 takes into account that the probe beam experiences a double pass through the film before reaching the light detector, but neglects possible interference effects. The rather broad bandwidth (10 nm) and spatial divergence of the probe beam is expected to smear interference effects effectively. The transition moment of the non degenerate 860 nm transition (the first excited singlet of $(BChl)_2$) is believed to lie normal to the reaction center cylindrical axis [31,52], i.e., in the plane of our Langmuir–Blodgett films. For an unpolarized probe beam this orientation results in the relationship $f = \frac{3}{4}(1 + \cos^2\beta)$, where β is the angle of refraction inside the reaction center film. Since $\theta = 45°$ and the index of refraction of the reaction center film is 1.32 [52], one concludes that $\beta = 32°$. It is straightforward to show that the electron-displacement distance projected along the film normal (and hence along the reaction center axis [31]) is given by

$$\Delta x = \frac{\epsilon_0 \epsilon_r \Delta V_{max}(0)}{e \delta N_{RC}}, \tag{19}$$

where ϵ_0 is the permittivity of vacuum, ϵ_r is the relative dielectric constant of the reaction center protein layer, and e is the fundamental charge. Assuming $\epsilon_r = 3$, a typical value for protein-type polymers [44,45], one obtains a value for the distance between $(BChl)_2$ and Q_A, projected onto the normal to the plane of the membrane (Δx) of 2.1 nm and 1.9 nm for the film samples A and B. However, in view of the effects neglected in deriving Eqn. 18 as discussed above, and the uncertainty in the precise value of ϵ_r, the values obtained are in good agreement with other measurements of the $(BChl)_2$-Q_A separation. Both EPR measurements [53] and electrical measurements [26] on reconstituted reaction center membrane multilayers suggest that $\Delta x > 1.5$ nm. Measurements [32] of photo-induced electrical transients on chromatophores, in the nanosecond time domain, gave $\Delta x \approx 2.4$ nm.

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