PHOTOELECTRIC CURRENTS ACROSS PLANAR BILAYER MEMBRANES CONTAINING BACTERIAL REACTION CENTERS

Response under Conditions of Single Electron Turnover

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ABSTRACT Light-induced electric current and potential responses have been measured across planar phospholipid membranes containing reaction centers from the photosynthetic bacterium *Rhodopseudomonas sphaeroides*. Under conditions in which the reaction centers are restricted to a single electron turnover, the responses can be correlated with the light-induced electron transfer reactions associated with the reaction center. The results indicate that electron transfer from the bacteriochlorophyll dimer to the primary ubiquinone molecule, and from ferrocytochrome *c* to the oxidized dimer occur in series across the planar membrane. Electron transfer from the primary to secondary ubiquinone molecule is not electrogenic.

INTRODUCTION

The reaction center (RC) of *Rhodopseudomonas sphaeroides* is an intracytoplasmic (chromatophore) membrane protein, and a constituent of the photosynthetic electron transfer cycle. Absorption of a photon by the RC drives an electron from a bacteriochlorophyll dimer, $(BChl)_2$, to a bacteriopheophytin, BPh. This charge separation is stabilized by secondary electron transfer from the BPh^- to the primary ubiquinone-10 molecule, Q_I, and subsequently from Q_I^- to Q_{II}. The oxidized $(BChl)_2^+$ can be re-reduced by a back reaction from either Q_I^- or Q_{II}^-, depending on the availability of Q_{II} to accept the electron from Q_I^-. Both back reactions are prevented by the presence of *c*-type cytochromes which catalyze a submillisecond re-reduction of $(BChl)_2^+$. The RC-catalyzed electron transfer reactions are summarized in Fig. 1 (1–3).

The RC spans the membrane (4, 5); the quinone-binding sites and cytochrome *c* adsorption are thought to be located on opposing sides of the membrane. Light-driven electron transfer is therefore coupled to the generation of a transmembrane electric current and potential. A direct measurement of the light-induced electric potentials and currents is prevented by the small size of the chromatophore vesicle (diameter 400 Å). Optical probes, however, such as the carotenoid bandshift, have been used as indicators of the membrane potential (6–11). Carotenoid band shifts arising from two of the electron transfer events associated with the RC have been resolved (8). Flash activation causes a fast band shift, which registers the charge separation between the $(BChl)_2$ and Q_I, whereas a slower phase is associated with the re-reduction of $(BChl)_2^+$ by ferrocytochrome c_2. To account for this observation it was proposed that electron transfer from $(BChl)_2$ to Q_I and from ferrocytochrome c_2 to $(BChl)_2^+$ occur in series across the chromatophore membrane (8).

The measurement of electric potentials using the carotenoid band shift may be unreliable because the probe may register local or surface potentials. Furthermore, the carotenoid band shift cannot be used to examine directly the current associated with electron transfer across the chromatophore membrane. It is therefore instructive to incorporate the RC into artificial membrane systems to allow a direct measurement of the light-induced electric response.

Recently, such measurements of both potentials and current generated by light-driven electron transfer have been made across planar phospholipid membranes containing RC (12–16), and across RC monolayers deposited between metal electrode films (16, 17). In this report, we describe the current and potential responses of planar RC membranes under conditions in which the RC are restricted to a single electron turnover. Our objective is to correlate the observed electric responses with the individual electron transfer reactions that are associated with

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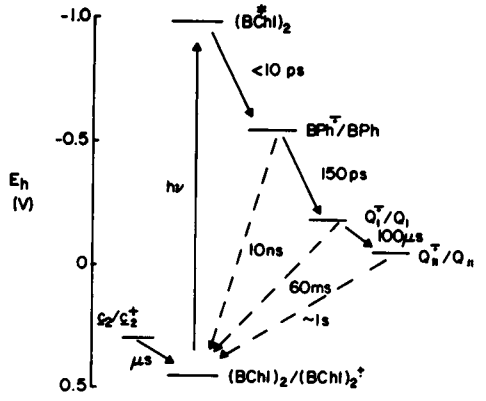

FIGURE 1 Energy profile of the electron transfer reactions catalyzed by the RC. The absorption of a photon by the RC drives an initial charge separation between the $(BChl)_2$ and a BPh. Subsequent electron transfers from BPh^- to Q_I and from Q_I^- to Q_{II} occur with half-times of 150 ps and 100 μs, respectively. The oxidized $(BChl)_2^+$ draws an electron from a *c*-type cytochrome. All times shown are half-times. The broken lines represent the back reactions to $(BChl)_2^+$ possible only if the next forward reaction is blocked, and if the $(BChl)_2^+$ has not been reduced by a cytochrome *c*. The products of the light reaction, ferricytochrome *c* and the ubisemiquinone, Q_{II}^-, do not recombine for several minutes. BPh^-, Q_I^- and Q_{II}^- represent the anionic radical states formed by single electron reduction of the individual species, the $(BChl)_2^+$ and c^+ represent the oxidized states formed upon the transfer of a single electron. The oxidation-reduction midpoint potentials for several of the RC components are uncertain. The loss of Q_I from the RC has no effect on the charge separation between the $(BChl)_2$ and BPh. However, the 10-ns charge recombination is faster than our instrument time constant, and no current transients driven by electron transfer from $(BChl)_2$ to BPh are detected.

the RC. In particular, we address the question of whether electron transfer from Q_I^- to Q_{II} and from ferrocytochrome *c* to $(BChl)_2^+$ can add to the light-induced membrane potential and current integral. The electric responses of the RC membranes under conditions of multiple electron transfer will be discussed elsewhere.[1]

MATERIALS AND METHODS

Preparation of RC and Phospholipid

RC containing Q_I but little or no Q_{II} were isolated from the carotenoidless R26 mutant of *Rps. sphaeroides* according to the procedure of Clayton and Wang (18). The reaction centers were kept in 10 mM Tris (hydroxymethyl) aminomethane-HCl, 0.1% lauryldimethylamine-*N*-oxide at −80°C until use.

Egg phosphatidylcholine and bovine brain phosphatidylserine were prepared according to procedures described in references 19 and 20, respectively. Egg phosphatidylethanolamine was obtained from Supelco, Inc. (Bellefonte, Pa.). All phospholipids were stored in chloroform under nitrogen at −80°C until use.

Transfer of RC from a Detergent to an Octane-Phospholipid Solution

The RC were transferred to an octane-phospholipid solution according to the procedure employed by Das and Crane (21) for the transfer of cytochrome *c* into an iso-octane solution. RC (0.4 mg protein/ml) were sonicated with phospholipid (10 mg/ml) in 10 mM 3-(*N*-morpholino) propanesulfonic acid (MOPS), pH 7.0, until the suspension became optically clear. The phospholipids used were phosphatidylcholine (PC), -ethanolamine (PE), and -serine (PS) in a 3:3:1 molar ratio. $CaCl_2$ (final concentration 100 mM) and 2 ml octane were added to the RC-phospholipid suspension and the mixture vortex-stirred for 4 min. Low-speed centrifugation separated the aqueous and alkane phases, and the alkane phase containing RC and phospholipid was retained. In some experiments, the octane phase was centrifuged at 104,000 *g* for 45 min to pellet the RC-phospholipid. This second centrifugation step rids the preparation of any quinone dislodged from the RC during the extraction (15, 22, 23).

Spectrophotometric measurements have shown that the alkane-solubilized RC retain their characteristic spectral and photochemical properties (15, 22, 23). The extraction procedure, however, incurs a loss (~50%) of Q_I binding. Addition of ubiquinone-10 (Sigma Chemical Co., St. Louis, Mo.) to the membrane-forming solution, followed by brief sonication (5 s), reconstitutes Q_I binding, and also serves to reconstitute partial Q_{II} binding (15, 22, 23). RC membranes were formed from either RC-phospholipid-octane solutions supplemented with ubiquinone-10 or from solutions without extra quinone.

We determined the RC concentration in the membrane-forming solution spectrophotometrically from the $(BChl)_2$ absorption at 865 nm, using an extinction coefficient of 126 $mM^{-1}\ cm^{-1}$ (24).

Detection of Light-induced Currents and Potentials Across Planar RC membranes

Measurements were made in a simple cell (25) consisting of a Teflon beaker in a perspex outer container. Lipid films were formed by blowing an aliquot of the membrane-forming solution across a 1-mm Diam hole punched in a thinned wall section of the beaker. The lipid films thinned to form uniform membranes that were either black, or silvery to reflected light. When black, such membranes displayed resistance values of 10^7–$10^8\ \Omega \cdot cm^2$, and capacitance values of 0.5–06. $\mu F/cm^2$.

Light activation was from either a xenon-flash (50-μs half peak width) or a mercury-arc lamp filtered through 1 cm of water, directed onto the RC membrane with a light guide. For steady-state light activation, a fast (1-ms time constant) electrical shutter (Uniblitz 23X) linked to a pulse generator controlled the duration of the light pulse. Electrical measurements were recorded using two silver-silver chloride electrodes connected either to a voltage clamp to monitor the current at constant potentials, or to a high impedance amplifier to monitor the potential. Both electrodes were shielded from the light. The output from the clamp or amplifier was displayed on either a storage oscilloscope (Tektronix, Inc., Beaverton, Ore., model 7613), or on a Digital oscilloscope (Nicolet Explorer 111A, Nicolet Instrument Corp., Madison, Wis.) interfaced to a PDP 11/10 computer system (Digital Equipment Corp., Maynard, Mass.). In some experiments the current records were stored and averaged by computer.

Our previous report (15) showed that the action spectrum of the light-induced current response of RC membranes matched the absorption spectrum of the RC in the RC-phospholipid-octane suspension.

All the work reported here was done without the removal of atmospheric oxygen. Under these conditions it was found that ~10–30% of the $(BChl)_2$ complement was in an oxidized state after membrane formation. These RC did not contribute to the light-induced electric current and potential responses. In many experiments no steps were taken to correct this loss of photocurrent activity. However, when investigating the contribution of electron transfer from ferrocytochrome *c* to $(BChl)_2^+$ to the light-induced current response, the problem became significant. Addition of ferrocytochrome *c* (Sigma Chemical Co.) to one aqueous phase results in the reduction of the $(BChl)_2^+$ accessible to that side of the membrane but not the other; this alone would cause the observation of a net current and potential after light activation. In these experiments, therefore, the oxidized $(BChl)_2$ were reduced, before cytochrome *c* was added, by the presence of sodium ascorbate in one or both aqueous

[1]Packham, N. K., P. L. Dutton, and P. Mueller. Manuscript in preparation.

phases. A sufficient amount of sodium ascorbate (100 μM) was added to maintain the $(BChl)_2$ in a reduced state, but not to permit multiple electron transfer in the light.

RC devoid of Q_I do not contribute to the light-induced electric responses, because the back reaction from the reduced BPh to the oxidized $(BChl)_2$ is too fast to be detected by our instruments.

RESULTS

Experimental Conditions Used to Limit the RC to a Single Electron Turnover

Planar bilayer RC membranes, formed from a uniform RC solution, contain two equal but oppositely directed RC populations (Fig. 2 *A*). Since the vectorial electron transfer reactions in the two RC populations cancel, light activation of the RC membrane does not elicit an electric response. To obtain a light-induced electric response, one RC population within the RC membrane must be inactivated before light excitation. This can be achieved by the addition of potassium ferricyanide to one aqueous phase, which causes the oxidation of the $(BChl)_2$ complement of one RC population. The ferricyanide apparently does not diffuse across the RC membrane, and the redox state of the $(BChl)_2$ complement accessible to the opposite aqueous phase is unaffected. In these light-activated RC, a single electron turnover is achieved by the omission of either extra ubiquinone or ferrocytochrome *c*. These conditions are summarized in Fig. 2.

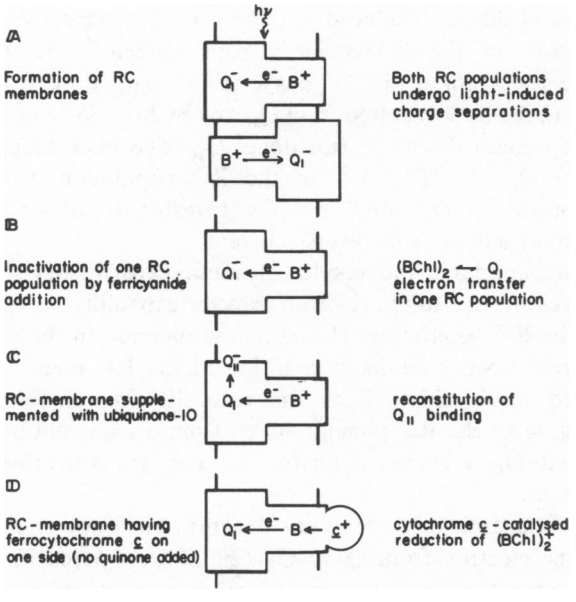

FIGURE 2 Experimental conditions used to obtain a single net $(BChl)_2$ oxidation upon steady state light activation of RC membranes. (*A*) both RC populations undergo light-driven electron transfer from $(BChl)_2$ to Q_I. No net electric current or potential responses are obtained. (*B*) one RC population inactivated prior to light excitation. In the absence of ferrocytochrome *c* and Q_{II}, light-induced electric current or potential responses are due to charge separation between $(BChl)_2$ and Q_I in the opposing RC population. (*C*) RC membrane supplemented with ubiquinone-10. Added quinone restores full Q_I binding, and partially reconstitutes Q_{II} binding. With no ferrocytochrome *c* added, there is only a single electron transfer from the $(BChl)_2$ to Q_{II}. (*D*) RC membrane with ferrocytochrome *c* added to one aqueous phase. In the absence of Q_{II} only one electron is transferred, because Q_I can only be singly reduced (31).

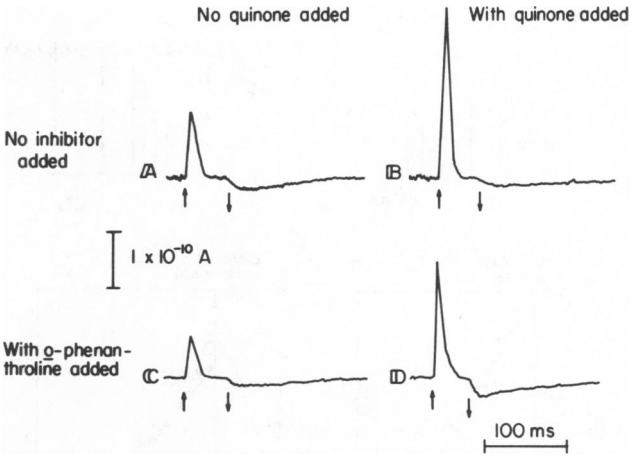

FIGURE 3 The light-induced electric current responses of the RC membrane: effect of ubiquinone-10 and *o*-phenanthroline. RC membranes (the membrane-forming solution had a $(BChl)_2$ concentration of 7.0 μM and was either devoid of (*A*, *C*) or supplemented with (*B*, *D*) 450 μM ubiquinone-10) were formed in 0.1 mM NaCl, pH 6.0. Potassium ferricyanide (2 mM) was added to one aqueous phase. The upward- and downward-directed arrows represent the onset and cessation, respectively, of illumination from a mercury-arc lamp. For traces *C* and *D*, 1 mM *o*-phenanthroline was added as an ethanol solution to the ferricyanide-containing aqueous phase. All traces shown are an average of 20 current responses spaced 2.5 s apart.

Light-induced Current Response

Electron Transfer from $(BChl)_2$ to Q_I. Fig. 3 *A* shows the steady-state light-induced response of RC membranes that are devoid of added quinone, and in which the electron transfer reactions are restricted to the $(BChl)_2$-to-Q_I charge separation. The experimental conditions correspond to scheme *B* of Fig. 2.

The light-induced current response of Fig. 3 *A* consists of a transient peak current that relaxes with first-order kinetics to zero current. A transient reverse current of opposite polarity is detected upon the cessation of illumination. The direction of the peak current is consistent with a light-driven charge separation from $(BChl)_2$ to Q_I in those RC whose $(BChl)_2$ remain inaccessible to the ferricyanide. Both the extent and the subsequent relaxation rate of the peak current depend on the light intensity (not shown), which indicates that the rate-limiting step is the light-driven $(BChl)_2$ oxidation.

The dark reverse current of Fig. 3 *A* has a relaxation half-time of 60 ms. An identical half-time has been determined for the back reaction, Q_I^- to $(BChl)_2^+$, in alkane-solubilized RC (15, 22) and chromatophore membranes (26, 27). The time-course of the back reaction from Q_I^- can be also obtained from a study of the kinetics with which the $(BChl)_2$ regain photocurrent activity between two light pulses. Fig. 4 *A* and *B* shows that the recovery of

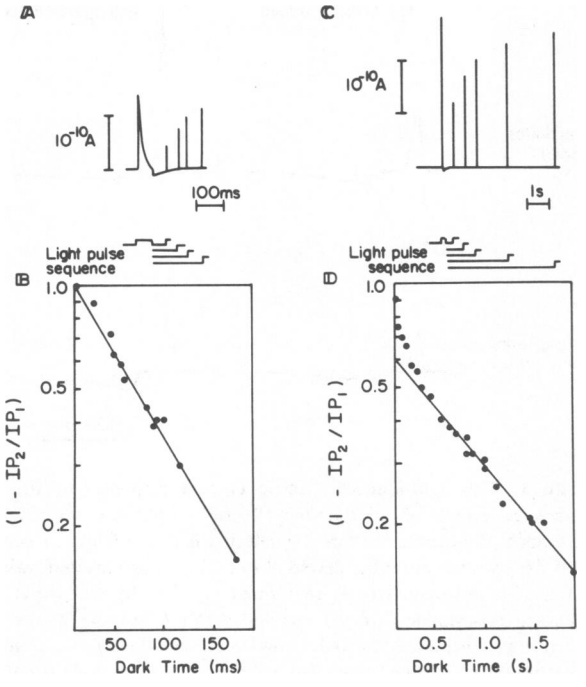

FIGURE 4 The recovery kinetics of photocurrent activity of RC membranes in the absence (*A*, *B*) and presence of added ubiquinone (*C*, *D*). The RC membranes (the membrane-forming solution had a $(BChl)_2$ concentration of 7.0 μM and was either devoid of (*A*, *B*) or supplemented with (*C*, *D*) 450 μM ubiquinone-10) were formed in 0.1 mM NaCl, pH 6.0. Ferricyanide (2 mM) was added to one aqueous phase before light activation. (*A*, *B*) light-induced current responses upon repetitive double light pulse sequences. The individual sequence consisted of two light pulses of 50-ms duration separated by a variable dark time. Shown are the complete current responses upon the first pulse, but only the initial peak current at the onset of the second pulse. The double pulse sequence was repeated after a 2-min delay. (*B*, *D*) recovery kinetics of the peak current. The peak current extent generated by the second light pulse (IP_2) was divided by the peak current extent generated by the first pulse (IP_1). The result was subtracted from 1.0, and plotted on a semilogarithm scale against the dark-time between the light pulses. In *D*, 10% of the peak current recovered with a slow half-time (in minutes). This phase was subtracted from the result.

photocurrent activity is first-order with a half-time of ~60 ms.

In summary, the peak current is due to the light-driven charge separation from $(BChl)_2$ to Q_I. The integrals of the peak and reverse currents match.

Electron Transfer from $(BChl)_2$ to Q_{II}. Spectroscopic measurements have shown that the addition of ubiquinone-10 to the membrane-forming solution results in an increased number of RC with a bound Q_I, and a partial reconstitution of Q_I^--to-Q_{II} electron transfer (15, 22, 23).

The results of Fig. 4 *C* and *D*, which were taken under the experimental conditions of scheme *C* of Fig. 2, show that addition of ubiquinone-10 to the RC membrane partially restores Q_{II} binding in the RC. A consequence of the reconstitution of Q_I^--to-Q_{II} electron transfer is that the subsequent back reaction from Q_{II}^- to $(BChl)_2^+$ is slower than that from Q_I^- to $(BChl)_2^+$; the back reaction from Q_{II}^- has reported half-times of 1–10 s in detergent-solubilized RC (28) and chromatophore membranes. Therefore, the reconstitution of Q_{II} binding in RC membranes can be deduced from the recovery kinetics of photocurrent activity between light pulses. In Fig. 4 *C*, the recovery of the peak current does not follow first-order kinetics. A semilogarithmic plot (Fig. 4 *D*) reveals two distinct phases in the recovery. Approximately 30% of the peak current recovers with a 60-ms half-time, which indicates the contribution of RC remaining devoid of Q_{II}. The remaining 70% represents the RC in which the Q_I^--to-Q_{II} electron transfer capability is reconstituted. Approximately 90% of this RC population containing Q_{II} recovers photocurrent activity with a half-time of 1 s, the remainder[2] shows a half-time much longer than 1 s.

The light-induced current response of the RC membranes supplemented with ubiquinone is shown in Fig. 3 *B*. When compared with RC membranes devoid of added quinone (Fig. 3 *A*), the effect of extra quinone on the current response is readily observed. We note that the light-induced peak current is increased and the dark reverse current attenuated, although the relaxation time constant of both the peak current and the reverse current remain unaffected. In contrast to Fig. 3*A*, the integral of the dark reverse current of Fig. 3*B* does not match the value of the light-induced peak current; it is typically only 30–40% of the light-induced peak current. The dark reverse current of Fig. 3 *B* has a half-time of 60 ms, and is due to the back reaction from Q_I^- to $(BChl)_2^+$ in those RC that remain devoid of functional Q_{II}. The back reaction from Q_{II}^- to $(BChl)_2^+$, in the RC population with a reconstituted Q_I^--to-Q_{II} electron transfer, is too slow to promote a detectable reverse current.

In summary, the results of these experiments show successful Q_I^--to-Q_{II} electron transfer capability in ~70% of the RC population. However, the increase in the peak current upon ubiquinone addition to the RC membrane could result either from the reconstitution of full Q_I binding to the RC population, or from a contribution of Q_I^--to-Q_{II} electron transfer to the transmembrane current.

o-Phenanthroline has been shown to block the transfer of the electron from Q_I to Q_{II} (29, 30). As a result, the back reaction from Q_I^- to $(BChl)_2^+$ is promoted in RC devoid of cytochrome *c* (26, 27). The inhibitor blocks the same reaction in the RC membranes, as demonstrated by its effect on the dark reverse current transient in membranes containing Q_{II}. As mentioned above, in RC

[2]This population probably results from the reoxidation of Q_{II}^- by interaction with the accessible ferricyanide, or with molecular oxygen. In the experiment of Fig. 4, the pulse duration was 50 ms. With RC membranes supplemented with ubiquinone-10, light pulses of longer duration result in a larger proportion of the RC recovering photocurrent activity with a half-time longer than 1 ms.

membranes supplemented with ubiquinone the dark reverse current integral is smaller than the peak current integral, because the back reaction from Q_{II}^- to $(BChl)_2^+$ is not detected as a reserve current. In Fig. 3 *D*, where *o*-phenanthroline was added to the ferricyanide-containing aqueous phase, the dark reverse current integral matched the light-induced peak current integral. The increase in the dark reverse current integral is due to an increased number of Q_I^- that remain unoxidized by Q_{II}. The back reaction from Q_I^- to $(BChl)_2^+$ with the characteristic 60-ms half-time is thereby promoted. *o*-Phenanthroline has no effect on the reverse current of RC membranes devoid of Q_{II} (compare Figs. 3 *A* and *C*).

Supplementary to its inhibition of the Q_I^--to-Q_{II} electron transfer, which can be detected immediately upon inhibitor addition to the aqueous phase, there is a second effect on the light-induced current response caused by *o*-phenanthroline. On a timescale of minutes the inhibitor causes an attenuation of the light-induced peak current. This effect is independent of its inhibition of the Q_I^--to-Q_{II} electron transfer; for example, the effect can be seen (compare Figs. 3 *A* and *C*) in RC membranes devoid of added quinone and therefore without Q_{II}.

Fig. 5 shows the inhibitory effect of *o*-phenanthroline on RC membranes in the absence of ferricyanide. The experimental situation is similar to that of scheme *A* of Fig. 2, except for the addition of ubiquinone-10. With no *o*-phenanthroline added, light activation does not elicit a net electric current response (Fig. 5 *A*). After addition of *o*-phenanthroline to one aqueous phase, light activation again does not induce a peak current transient (Fig. 5 *B*). However, a reverse current of 60-ms half-time is observed upon the cessation of the light. The detection of the reverse current indicates that the back reaction, Q_I^- to $(BChl)_2^+$, is promoted in RC whose Q_I^--to-Q_{II} electron transfer is blocked by *o*-phenanthroline. In the opposing RC population, whose quinone binding sites are inaccessible to the inhibitor, Q_I^--to-Q_{II} electron transfer is presumably uninhibited and the slow back reaction from Q_{II}^- to $(BChl)_2^+$ unaffected. The absence of a peak current in Fig. 5 *B* demonstrates that the light-induced current amplitudes in the two opposing RC populations remain unaltered by the action of *o*-phenanthroline. Therefore, electron transfer from Q_I^- to Q_{II}, which is blocked in one RC population, does not contribute to the forward current.

We conclude that the enhanced light-induced current in the presence of added quinone (Fig. 3 *B*) is due to the reconstitution of full Q_I binding, with the result that there is an increased number of RC capable of stabilizing the charge separation between the $(BChl)_2^+$ and BPh^-. Electron transfer from Q_I^- to Q_{II} is electronically silent, and does not contribute to the light-induced electric current and potential responses.

Ferrocytochrome c-to-$(BChl)_2^+$ Electron Transfer. Fig. 6 shows the effect of adding ferrocytochrome *c* to one side of the RC membrane. In these experiments ascorbate (0.1 mM) was present to make certain that the $(BChl)_2$ complement was reduced before ferrocytochrome *c* was added (see Methods). With ascorbate present in both aqueous phases, no light-induced current is detected (Fig. 6 *A*). Addition of ferrocytochrome *c* to one aqueous phase promotes a current response after flash activation. (Fig. 6 *B*). The polarity of the current response is consistent with electron transfer from the side containing the added ferrocytochrome *c*. This result indicates that the rereduction of $(BChl)_2^+$ by ferrocytochrome *c* generates a transmembrane electric current. Fig. 6 *C* provides support for this conclusion. Addition of protamine sulfate, a highly polar molecule that is expected to lower the effective cytochrome *c* concentration at the RC membrane inter-

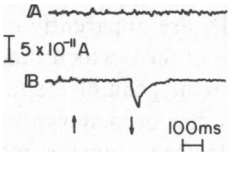

FIGURE 5 The light-induced electric currents in the absence (*A*) and presence (*B*) of *o*-phenanthroline. RC membrane (the membrane-forming solution had a $(BChl)_2$ concentration of 12 μM, and was supplemented with 450 μM ubiquinone-10) was formed in distilled water, pH 6.0. No ferricyanide was added. Illumination was from a mercury-arc lamp; the upward- and downward-directed arrows represent the onset and cessation of illumination, respectively. (*A*) No further additions. (*B*) 1 mM *o*-phenanthroline added to one aqueous phase.

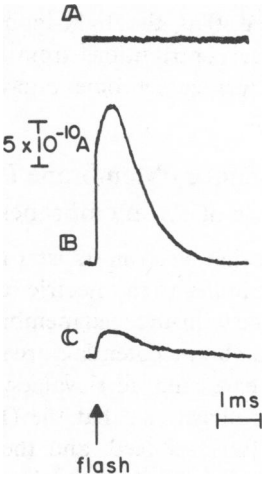

FIGURE 6 Flash excitation of RC membranes supplemented with ubiquinone-10: effect of ferrocytochrome *c* and protamine sulfate on the current response. RC membrane (the membrane-forming solution had a $(BChl)_2$ concentration of 10.4 μM, and supplemented with 450 μM ubiquinone-10) was formed in 4 mM NaCl, pH 6.0. Sodium ascorbate (100 μM) was added to both aqueous phases. Light activation was provided by xenon-flash. An electrical artifact was recorded upon the discharge of the flash power supply. Each record shown is corrected for the electrical artifact. (*A*) No further additions. Light activation did not generate a current response (see text). (*B*) Ferrocytochrome *c* (25 μM) added to one aqueous phase. The current response is due to the cytochrome *c* catalysed reduction of the $(BChl)_2^+$ complement in one RC population. (*C*) As in (*B*), but with protamine sulfate (2 mg total) added to the aqueous phase containing cytochrome *c*.

face and thereby slow the electron transfer reaction from ferrocytochrome *c* to $(BChl)_2^+$, abolishes the current that was promoted by the presence of cytochrome *c*.

The experiments of Fig. 6 were done with RC membranes supplemented with ubiquinone-10 to ensure a full complement of Q_I. Therefore, with ferrocytochrome *c* added, there is the possibility of multiple turnovers during illumination. It is unlikely, however, that this is the reason for the cytochrome *c*-promoted current response because: (*a*) Ferrocytochrome *c* addition to RC membranes devoid of extra quinone has been shown to result in an increased light-induced peak current integral (16). Under these conditions, which are comparable to scheme *D* of Fig. 2, the RC are restricted to a single turnover because Q_I^- blocks further photochemistry (31). (*b*) Addition of *o*-phenanthroline, which also restricts the system to a single electron turnover, has only a negligible effect on the light-induced current response (data not shown).

In both experimental situations, strong evidence that the RC were restricted to a single net $(BChl)_2$ oxidation comes from the observation that the second and subsequent flashes yielded no further current response. In this situation, the back reaction from Q_I^- to $(BChl)_2^+$ is prevented by a much faster transfer of an electron from ferrocytochrome *c* to $(BChl)_2^+$. The Q_I^- blocks further photochemistry. The subsequent return of the light-induced current response in both experimental stations required a dark half-time of many minutes, and presumably depended upon the reoxidation rate of Q_I^-.

It is concluded that the light-induced peak current response can have contributions from both the $(BChl)_2$-to-Q_{II} and the ferrocytochrome *c*-to-$(BChl)_2^+$ electron transfer reactions.

Light-induced Membrane Potential Response of RC membranes

Further evidence that electron transfer from cytochrome *c* to $(BChl)_2^+$ contributes to the electric response is obtained from a study of the light-induced membrane potential. The flash-induced membrane potentials are shown in Fig. 7. In this case, ascorbate and ferricyanide were present in opposite aqueous phases; so that the $(BChl)_2$ of one RC population was fully reduced, and the other population fully oxidized, before flash activation. In these experiments the RC membranes were supplemented with ubiquinone to ensure a full complement of Q_I.

Fig. 7 *A* shows the light-induced membrane potential across RC membranes in which the electron transfer reactions are restricted to charge separation between $(BChl)_2$ and Q_{II}. The potential has a value of ~1 mV. Addition of ferrocytochrome *c* to the ascorbate-containing aqueous phase causes nearly a twofold increase in the membrane potential elicited by light activation (Fig. 7 *A*). Addition of *o*-phenanthroline had no immediate effect on the membrane potential—a result consistent with the

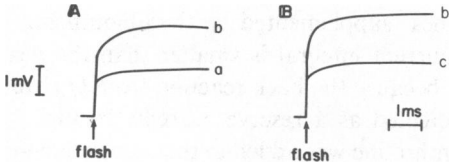

FIGURE 7 Flash excitation of RC membranes supplemented with ubiquinone-10: effect of ferrocytochrome *c* and protamine sulfate on the membrane potential response. RC membrane (the membrane-forming solutions had a $(BChl)_2$ concentration of 11.6 μM and supplemented with 450 μM ubiquinone-10) was formed in 4 mM NaCl. Potassium ferricyanide (2 mM) and sodium ascorbate (100 μM) were added to opposite aqueous phases. (*A*) Lower trace (*a*) obtained with no further additions. This response is due to the light-induced electron transfer from $(BChl)_2 \rightarrow Q_I$. The upper trace (*b*) was obtained with ferrocytochrome *c* (25 μM) added to the ascorbate-containing aqueous phase. This upper response is therefore due to the light-induced electron transfer between cytochrome $c \rightarrow (BChl)_2 \rightarrow Q_I$. (*B*) Upper trace (*b*) as upper trace (*b*) in (*A*). Lower trace (*c*) obtained with protamine sulfate (2 mg total) added to aqueous phase containing the cytochrome *c*.

conclusion of the previous sections that Q_I^--to-Q_{II} electron transfer is electrically silent, and that multiple turnovers of the RC are not occurring to any significant extent upon flash activation in the presence of cytochrome *c* and Q_{II}.

The addition of protamine sulfate abolishes the cytochrome *c*-induced membrane potential (Fig. 7 *B*). Protamine sulfate has no effect on the membrane potential generated by $(BChl)_2$-to-Q_I electron transfer.

These results are consistent with those obtained from the light-induced current responses, and with the interpretation that electron transfer from the $(BChl)_2$ to Q_I and the subsequent rereduction of $(BChl)_2^+$ by ferrocytochrome *c* occur in series across the RC membrane.

DISCUSSION

The procedures required to solubilize the RC into the octane-phospholipid suspension have little, or no effect on the $(BChl)_2$ or BPh complement of the RC (15, 22, 23). However, virtually all the Q_{II} and up to 50% of the Q_I are lost during the preparative procedures. The Q_I appears to be readily reconstituted by addition of ubiquinone-10 to the membrane-forming solution, but only 70% of the Q_{II} is reconstituted in a way that promotes Q_I^--to-Q_{II} electron transfer. The reason for this heterogeneity is not clear at present. In the RC membrane a small proportion (10–30%) of the $(BChl)_2$ are apparently oxidized before light activation. Addition of ferrocytochrome *c* or sodium ascorbate reduces this $(BChl)_2$ complement.

o-Phenanthroline has been shown to have two independent effects on electron transfer reactions in the RC membrane. The immediate result of adding *o*-phenanthroline to one aqueous phase is the inhibition of Q_I^--to-Q_{II} electron transfer in the RC population whose quinone binding sites are accessible to that side of the RC membrane. On a longer timescale there is an attenuation of the light-induced peak current, which is probably due to

the inhibition of Q_I reduction. This secondary effect of *o*-phenanthroline is observed also in RC membranes containing only Q_I (Fig. 3). It may be suggested that *o*-phenanthroline operates in the RC membrane (*a*) rapidly, by displacing Q_{II} from its functional binding site and thereby inhibiting Q_I^--to-Q_{II} electron transfer; and (*b*) on a much slower time scale, by displacing Q_I from its binding site and thus preventing the BPh^--to-Q_I electron transfer. This secondary effect seems to involve both RC populations, and implies that *o*-phenanthroline can cross the RC membrane.

Light-induced Current Response

The peak current has contributions from both $(BChl)_2$-to-Q_I and ferrocytochrome *c*-to-$(BChl)_2^+$ electron transfer reactions. The observation that electron transfer from Q_I^- to Q_{II} is electrically silent does not a priori resolve the position of Q_{II} in the RC membrane; the electrically silent step could either occur in the membrane plane or be normal to the membrane plane and accompanied by proton transfer.

The integral of the transient peak current, Q, is determined by the number of electrons transferred across the membrane and by the distance of the individual charge transfer steps.

A dark reverse current is observed only in RC membranes in which the light-induced electron transfer reactions are restricted to the $(BChl)_2$-to-Q_I charge separation. Under these conditions, the reverse current has a 60-ms half-time commensurate with the back reaction from Q_I^- to $(BChl)_2^+$, and the reverse current integral matches the peak current integral. When Q_{II} is present to accept the electron from Q_I^-, the reverse current is not detected because the back reaction from Q_{II}^- to $(BChl)_2^+$ is too slow ($t_{1/2}$ = 1 s, Fig. 4). No reverse current is observed when cytochrome *c* is present, because the back reaction from Q_I^- is blocked by the fast reduction of the light-oxidized $(BChl)_2$ by ferrocytochrome *c*.

Light-induced Potential Response

The light-induced current charges the planar RC membrane to a potential, V, whose magnitude depends on both the peak current integral, Q, and the capacitance, C: $V = Q/C$. The total capacitance C may be considered to comprise of two independent, but parallel capacities, a membrane capacity, C_M, and an RC capacity, C_{RC}.

Both the $(BChl)_2$-to-Q_I and the cytochrome *c*-to-$(BChl)_2^+$ electron transfer reactions occur across a region of low dielectric constant within the RC capacity. Fig. 8 shows a schematic representation of the RC embedded in the planar membrane. We assume that the low dielectric constant region of the RC is bounded by the aqueous phases. The Q_I is positioned at the terminus of the RC capacity, because there is no evidence that Q_I^--to-Q_{II} electron transfer contributes to the current.

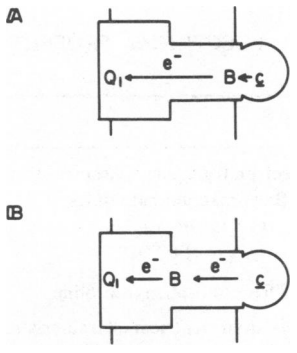

FIGURE 8 Schematic representation of the RC membrane. The reaction center is considered to span the membrane, with the primary ubiquinone molecule positioned on the opposite side of the membrane to the cytochrome *c* adsorption. The position of the $(BChl)_2$ is considered to be either at the region of the RC opposite to the Q_I binding site (*A*), or to be located towards the center of the RC (*B*). See text for further details.

In Fig. 8 we consider the differences in electric responses when the $(BChl)_2$ is positioned either at the opposite terminus of the RC capacity (Fig. 8 *A*), or toward the center of the RC capacity (Fig. 8 *B*). Light-driven electron transfer from $(BChl)_2$ to Q_I could occur either across the complete RC capacity, or across only one-half of the RC capacity, respectively. In both cases, cytochrome *c* is considered to be adsorbed to the RC at the membrane-water interface.

In Fig. 8 *A*, the rereduction of $(BChl)_2^+$ by ferrocytochrome *c* results in the transfer of an electron across the cyctochrome *c* molecule. This electron transfer would not elicit an electric response because counter-ion movement at the membrane interface would cancel any current response. If the cytochrome *c* were insulated from the aqueous phase, the cytochrome *c* adsorption would result in a decreased RC capacity. In this case, cytochrome *c* oxidation would theoretically increase the measured potential, but not the measured current integral.

In Fig. 8 *B*, electron transfer from the cytochrome *c* to the $(BChl)_2^+$ occurs across one-half of an unaltered RC capacity. In this case the charge movement is expected to increase both the measured current integral and membrane potential. The observation (Figs. 6 and 7) that cytochrome *c* oxidation increases both the current integral and potential supports the scheme of Fig. 8 *B*.

Summary of the Electrical Properties of the RC Membrane

Table I summarizes the electrical data obtained in this study. It includes the measured current and potential values, and compares them with the computed values obtained from the $(BChl)_2$ concentration and membrane capacitance. Where applicable, data from the chromatophore membrane are included.

The transfer of an electron across the RC capacity from the adsorbed cytochrome *c* to the Q_I will generate a peak

TABLE I
ELECTRICAL PROPERTIES OF THE RC MEMBRANE AND THE CHROMATOPHORE MEMBRANE

	RC membrane	Chromatophore
Electron transport characteristics		
Back reaction half-times:		
$-Q_I^-$ to $(BChl)_2^+$	60 ms	60 ms (26, 27)
$-Q_I^-{}_1$ to $(BChl)_2^+$	1 s	1–10 s (26, 28)
Effect of *o*-phenanthroline	Blocks Q_I^- to Q_{II} Displaces Q_I	Blocks Q_I^- to Q_{II} (29, 30)
Measured electrical characteristics		
Single turnover current integral	$2–10 \times 10^{-10}$ A · s/cm^{-2}	
Single turnover membrane potential	2 mV	70–100 mV (6–11)
Membrane capacitance	0.5–0.6 μF/cm^2	1.1 μF/cm^2 (11)
Membrane resistance	10^7–10^8 Ω · cm^2	10^6 Ω · cm^2 (32)
Computed electrical characteristics		
RC density	$1–6 \times 10^9$ RC/cm^2	2×10^{11} RC/cm^2 (11)
Computed membrane potential	0.5–2 mV	
Calculated peak current (for 150 ps electron transfer)	2 A/cm^2	

current, whose integral depends on the number of electrons traveling across the membrane and the distance of charge separation as a portion of the dimension of the nonconductive region of the RC. Under single turnover conditions, light activation of RC membranes supplemented with cytochrome *c* generates peak current integrals of $2–10 \times 10^{-10}$ A · s/cm^2, corresponding to the transfer of $1–6 \times 10^9$ electrons/cm^2 across the RC capacity.

The RC membranes were formed from a membrane-forming solution that had $(BChl)_2$ concentrations of 7–14 μM. Assuming a membrane thickness of 60 Å (33), we calculate that $1.3–2.6 \times 10^9$ RC/cm^2 were present in the membrane with their $(BChl)_2$ complement accessible to one aqueous phase.

The onset of steady state illumination generates a peak current whose initial extent and subsequent relaxation rate is determined by the light intensity. Direct measurements of the light intensity yield values of ~100 photons/RC·s. Similar intensity estimates can be deduced from the relaxation time constant of the peak current. With the membrane of Fig. 3 *B*, the maximal current in response to a saturating picosecond laser pulse would be 2 A/cm^2; limiting step would be the 150-ps electron transfer from the BPh^- to Q_I. The instrumentation time constant prevents measurements of such brief currents.

Current and potential simulations derived from a quantitative description of the electron transfer reactions associated with the reaction center match the light-induced responses.[3]

The light-induced potential of 2 mV, observed in Fig. 7, is consistent with the measured charge integral and membrane capacitance. The magnitude of the light-induced potential is limited by the number of reaction centers present within the membrane. In the chromatophore membrane, across which single turnover potentials of ~100 mV have been calculated from carotenoid band shift data (6–11), the average RC density is 2×10^{11} RC/cm^2 (11). The difference in the determined light-induced potentials between the chromatophore and RC membrane is due to the ~100-fold larger RC density in the native photosynthetic membrane. Thus, the agreement between the two measurements suggests that the carotenoid band shift can be used as an empirical indicator of the transmembrane electric potential across the bacterial chromatophore.

[3]Mueller, P., P. L. Dutton, and N. K. Packham. Unpublished observations.

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