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Orientation of Reaction Center Complexes from *Rhodobacter sphaeroides* in Proteoliposomes and the Effect of *o*-Phenanthroline on Electrogenesis During Primary Photochemical Reaction

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Abstract

The orientation of *Rhodobacter sphaeroides* reaction center complexes (RC complexes) in proteoliposomal membranes was investigated by a direct electrometric method. Conditions were found that allow monitoring of only that RC complex fraction that is oriented with its donor side to the inner part of the proteoliposome. It is shown that *o*-phenanthroline, an inhibitor of electron transfer between primary (Q_A) and secondary (Q_B) quinone acceptors, can also inhibit the photoinduced Q_A reduction. The efficiency of this inhibition depends on the concentration of added ubiquinone. It is assumed that the laser flash-induced *o*-phenanthroline inhibition of primary dipole ($P\text{-}870^+ \cdot Q_A^-$) formation is of a competitive nature.

Key Words: *Rhodobacter sphaeroides*; reaction center complexes; proteoliposomes; *o*-phenanthroline.

Introduction

Reaction center complexes (RC complexes) have been established as a photoelectric generator in chromatophores of photosynthetic bacteria (Jackson and Crofts, 1971; Drachev *et al.*, 1975). Isolated RC complexes reconstituted in proteoliposomes and incorporated into planar phospholipid membrane proved to be a useful model for studies on the mechanism of electric potential difference ($\Delta\Psi$) generation by these pigment–protein complexes (Drachev *et al.*, 1976, 1984a). Illumination of such systems

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containing RC complexes from *Rhodobacter sphaeroides* was found to produce some electric potential difference across the planar membrane (Drachev *et al.*, 1984a). However, the low amplitude of $\Delta\Psi$ in these proteoliposomes, by contrast with chromatophores (Drachev *et al.*, 1984b), limited the kinetic analysis. We have assumed that the low value of the photoresponse can be explained by the random orientation of the RC complexes in such types of proteoliposomes (Iba *et al.*, 1984). The existence of differently oriented populations of RC complexes does not allow an unambiguous interpretation of the kinetics of the laser flash-induced $\Delta\Psi$ generation in the "proteoliposome–collodion phospholipid membrane" system.

In the present study, the orientation of *R. sphaeroides* RC complexes in proteoliposomal membranes was investigated by a direct electrometric method. Conditions were found that allow monitoring of only that RC complex fraction that is oriented with its donor side to the inner part of the proteoliposome. It was shown that *o*-phenanthroline can inhibit the photoinduced Q_A reduction.

Materials and Methods

Asolectin (phosphatidylcholine type 11S) and ubiquinone-10 (CoQ_{10}) were from Sigma, lauryldimethylamine *N*-oxide was from Fluka, and HEPES was from Serva. Ascorbic acid was purchased from Koch-Light. *o*-Phenanthroline was from Chemapol (Czechoslovakia). Cholic acid (Sigma) was recrystallized from 70% ethanol. Other reagents were commercial products of the highest purity available.

Chromatophores of *R. sphaeroides* (strain 1760-1) were isolated by an ultrasonic treatment procedure as described previously (Isaev *et al.*, 1970), and RC complexes were isolated from chromatophores according to Zakharova *et al.* (1981). Proteoliposomes were prepared by dialysis (Racker, 1972) as follows: a mixture containing asolectin (80 mg/ml) and cholate (4%) in 100 mM K^+-phosphate buffer (pH 7.5) was sonicated in 20 sec bursts (50 mA, 22 kHz) up to a total sonication time of 3 min in a UZDN-2T ultrasonic disintegrator. Then the clear solution was mixed with an equal volume of the RC complex preparation ($A_{870} = 1.5$) in 30 mK K^+-phosphate buffer (pH 7.3) containing 0.05% sodium cholate and sonicated for an additional 6–8 sec and dialyzed against 2000 volumes of 50 mM K^+-phosphate buffer (pH 7.5) for 26 h in the dark at 4°C. Proteoliposomes were centrifuged at 165,000 × *g* for 60 min, and the resulting pellet was suspended in a small volume of 50 mM K^+-phosphate buffer (pH 7.5). Proteoliposomes containing 2 mM ascorbate and 5 mM ferrocyanide were prepared in the presence of these compounds at every step of the procedure.

For incorporation of proteoliposomes into collodion film, the vesicles were introduced into one of the compartments of the Teflon cell containing 20 mM HEPES buffer (pH 7.5), separated by collodion film, impregnated by phospholipids (80 mg asolectin per ml decane), and incubated for 3 h in the presence of 30 mM $CaCl_2$. To remove the excess of $CaCl_2$ and proteoliposomes not bound to the membrane, both compartments of the cell were washed with 10 volumes of 20 mM HEPES buffer (pH 7.5) using a peristaltic pump. An OGM-40 laser (λ = 694 nm, 30 ns half-width, 20 mJ output) was used as a source of light pulses. Photoelectric activity was monitored electrometrically as the electric potential difference across the collodion film with a pair of Ag/AgCl electrodes connected via an operational amplifier (Burr Brown 3554 BM) and a transient recorder (Data Lab DL-922) with a NOVA 3D minicomputer (Data General). The resolution time of the system was 0.2 μsec as limited by DL-922 parameters.

Results and Discussion

Figure 1a shows typical laser flash-induced electric responses of RC complex-containing proteoliposomes associated with collodion film. The flash generates $\Delta\Psi$ of a sign which corresponds to positive charging of the interior of the vesicles. The polarity of light-dependent $\Delta\Psi$ coincides in all three types of preparations containing RC complexes from *R. sphaeroides*, namely, chromatophores, proteoliposomes containing purified RC complexes, and proteoliposomes with isolated antenna bacteriochlorophyll-containing

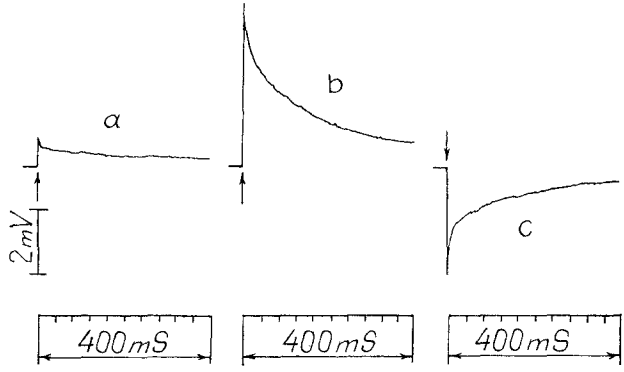

Fig. 1. Laser flash-induced electric responses of *R. sphaeroides* RC complex proteoliposomes associated with a phospholipid-impregnated collodion film. Here and below, vertical arrows indicate laser flashes. (a) Without additions; (b) +2 mM ferricyanide; (c) as (b) +4 mM ascorbate.

RC complexes (data not presented). Evidently, the electrogenic reaction catalyzed by RC complexes oriented with their donor side to the inner part of the proteoliposomes (internal P-870 fraction) only insignificantly predominates over the reaction catalyzed by oppositely oriented complexes (external P-870 fraction). This phenomenon accounts for the low amplitude of photoresponses in such proteoliposomes. To verify this assumption, a nonpenetrating oxidizing agent—ferricyanide—was added to the incubation mixture. Figure 1b shows that the addition of this agent causes a significant growth of the $\Delta\Psi$ amplitude induced by the laser flash. This phenomenon can evidently be explained by the fact that, in the presence of ferricyanide, the external P-870 fraction is oxidized and ceases functioning, and the electrons are transferred only in oppositely oriented complexes.

After the addition of the ascorbate excess into the medium, the sign of the photoelectric response changes to the opposite and corresponds to the transfer of negative charges into the proteoliposomes (Fig. 1c). The addition of ascorbate prior to ferricyanide does not alter the $\Delta\Psi$ sign (data not presented). These findings indicate that, initially, some part of the RC complex is oxidized and is not excited by the laser flash. Evidently, ascorbate itself is not capable of reducing the external P-870 fraction due to the fact that the special pair is immersed in the hydrophobic part of the membrane while the membrane interior is practically impermeable to ascorbate during the experiment. At the same time, in the presence of the ascorbate excess, ferricyanide turns into the ferrocyanide form, which is capable of reducing the special pair of P-870 fraction while the internal P-870 fraction remains partially oxidized.

On the basis of the data obtained, an assumption can be made that the maximum $\Delta\Psi$ amplitude with a positive sign can be reached when the whole internal P-870 fraction is reduced and all the P-870 molecules of the opposite orientation are oxidized. To verify this assumption, proteoliposomes reconstituted in the presence of ferrocyanide and ascorbate were used in the next series of experiments. Figure 2a shows that, originally, $\Delta\Psi$ in these proteoliposomes is substantially higher than that in proteoliposomes that do not contain the reducing agent in their inner part (compare with Fig. 1a). The addition of ferricyanide into the incubation mixture (Fig. 2b) leads to a further increase in the $\Delta\Psi$ amplitude, while the addition of the ascorbate excess does not cause any changes in its polarity and only slightly lowers its amplitude (Fig. 2c). Therefore, it can be concluded that when both populations of RC complexes are completely reduced, the direction of the total photoelectric response is determined by the predominance of the RC complex molecules which are oriented with their donor side to the inner part of the proteoliposomes.

The $\Delta\Psi$ amplitude can also be elevated using ubiquinone-10. It has been shown earlier that association of chromatophores and proteoliposomes with

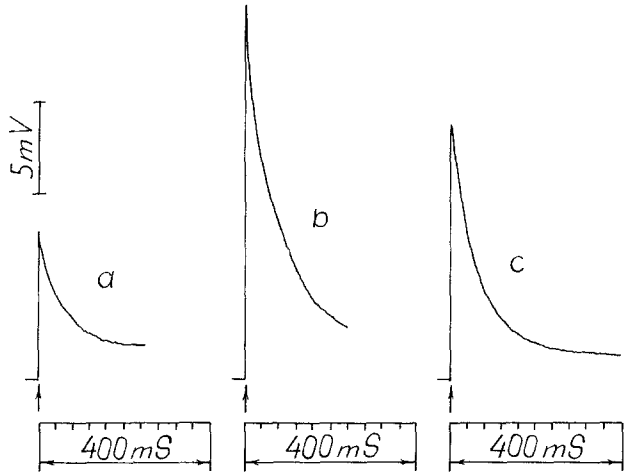

Fig. 2. Photoelectric responses in RC complex proteoliposomes containing 2 mM ascorbate and 5 mM ferrocyanide in the inner part. (a) Without additions; (b) +2 mM ferricyanide; (c) as (b) +4 mM ascorbate.

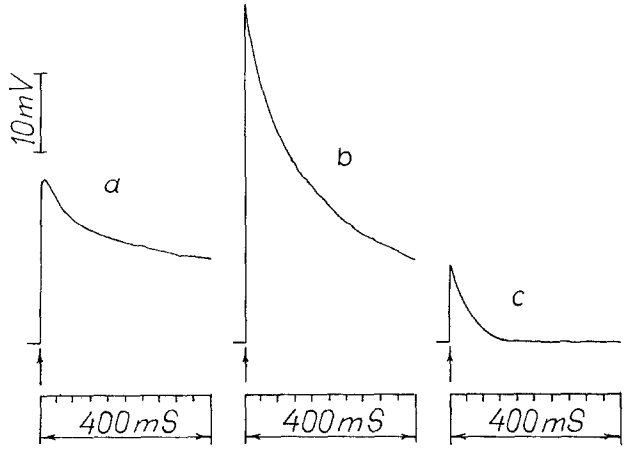

Fig. 3. Photoelectric responses in RC complex proteoliposomes. CoQ_{10} (15 mg/ml) was added to the decane solution of phospholipids used to impregnate the collodion film. (a) Without additions; (b) +2 mM ferricyanide; (c) as (b) +2 mM *o*-phenanthroline.

the phospholipid membrane or the collodion film saturated with phospholipids is accompanied by an extraction of secondary (Q_B) and partially primary (Q_A) quinone acceptors by decane which is used for the preparation of the solution of phospholipids (Drachev *et al.*, 1976, 1984). It seems likely that this defect of our system of measurements can be overcome by the addition of ubiquinone-10 into the decane solution. Figure 3 depicts the results of one

of the experiments with proteoliposomes containing ferrocyanide in their inner part. Saturation of the planar membrane by ubiquinone-10 (Fig. 3a) leads to a further rise in the photoelectric response (compare with Fig. 2a). The addition of ferricyanide causes a more than two-fold increase in the $\Delta\Psi$ amplitude, which, in this case, amounts to 45 mV (Fig. 3b). This photoresponse, in its turn, is almost two times higher than that in chromatophores from *R. sphaeroides* (Drachev *et al.*, 1981; Semenov *et al.*, 1984). This phenomenon can be explained by the higher density of RC complexes in proteoliposomal membranes in contrast to chromatophores. The possibility of concentrating the enzyme under study in one membrane and, consequently, to enhance the corresponding reaction can be regarded as one more advantage of the reconstitution method.

It has been found that under the above conditions, the amplitude of the photoresponse is practically completely determined by the formation of the primary dipole $P\text{-}870^+ \cdot Q_A^-$ in the RC complexes that are oriented by their donor side to the inner part of the proteoliposomes. In view of this, such a system seems to be the most suitable model for investigations of this process. In particular, it appeared of interest to enquire into the possible role of *o*-phenanthroline in RC complexes (Prince and Dutton, 1978). However, the previously obtained data indicate that *o*-phenanthroline not only can reversibly oust unstably bound secondary acceptor Q_B from its binding site on the protein (Wraight, 1981; Stein *et al.*, 1984) but may also inhibit the light-dependent reduction of Q_A (Wraight, 1981; Okamura *et al.*, 1975; Packham *et al.*, 1982). Still, the mechanism of action of this inhibitor awaits further study.

We have also investigated the quantitative parameters governing the *o*-phenanthroline effect on the electrogenic reaction of the primary dipole $P\text{-}870^+ \cdot Q_A^-$ formation in the above reconstitution system. Besides accelerating the photoelectric response decay brought about by the inhibition of the $Q_A \xrightarrow{e} Q_B$ reaction, 2 mM *o*-phenanthroline also causes a significant fall in the $\Delta\Psi$ amplitude in the membrane-associated proteoliposomes containing ferrocyanide (Fig. 3c). Figure 4 presents the results of quantitative titration of the photoelectric response by *o*-phenanthroline in reconstituted RC complexes at various concentrations of ubiquinone-10 in the decane solution of phospholipids. In the absence of ubiquinone-10, a 50% decrease in the $\Delta\Psi$ amplitude (I_{50}) is observed at an inhibitor concentration of 170 μM (curve 1). The stationary level of the photoresponse amplitude observed at saturating concentrations of the inhibitor amounts to 5% of the initial level. After the addition of ubiquinone-10 (15 mg/ml) into the phospholipid solution (curve 2), a relative decrease in the $\Delta\Psi$ amplitude is observed at higher concentrations of *o*-phenanthroline (I_{50} = 650 μM) Under the same conditions and at the same concentration of the inhibitor, the stationary level of

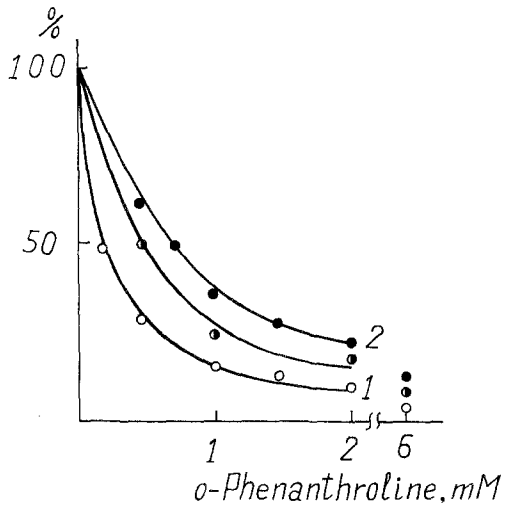

Fig. 4. Effect of *o*-phenanthroline on the amplitude of photoelectric responses in RC complex proteoliposomes without CoQ_{10} (○) and in the presence of 7 mg/ml (◐) and 15 mg/ml (●) CoQ_{10}. The conditions are similar to those described in the captions to Figs. 2 and 3.

$\Delta\Psi$ amounts to 12% of the initial level. It is highly improbable that this effect is caused by a positive shift in the redox potential of Q_A under the action of *o*-phenanthroline, since the amplitude of this shift is too small (less than 50 mV) to cause an inhibitory effect (Prince and Dutton, 1978). Therefore, the results obtained in the present study indicate that *o*-phenanthroline inhibits the primary photochemical reaction P-870 $\xrightarrow{e}$ Q_A and permit us to speculate on the competitive mechanism of this inhibition with respect to quinone. The data obtained also suggest a close similarity between the mechanisms of inhibition of the P-870 $\xrightarrow{e}$ Q_A and $Q_A \xrightarrow{e} Q_B$ reactions by *o*-phenanthroline.

Acknowledgments

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