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MEMBRANE POTENTIAL EFFECT ON NANOSECOND RECOMBINATION LUMINESCENCE IN *RHODOSPIRILLUM RUBRUM*

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1. Introduction

Some data indicate that the pigment–protein complex of the reaction center in purple bacteria spans the chromatophore membrane [1–7]. Primary conversion of light energy in the reaction centers involves electron transfer from the photoexcited bacteriochlorophyll dimer P870 to the primary stable acceptor Q_1, which occurs via an intermediate transient acceptor I, probably, bacteriopheophytin or a complex of P800 and bacteriopheophytin [8]. Electron transfer from P870 to Q_1, is transmembraneous, as follows in particular from the dependence of the intensity of a ms afterglow, resulting from emissive recombination of $P870^+$ and Q_1^-, on the transmembrane electric potential difference, $\Delta\Psi$ [9]. As for electron transfer from P870 to I, it is not clear whether this stage of charge separation is of electrogenic character.

Charge separation in the reaction centers with Q_1 pre-reduced was accompanied by an intense short-lived (lifetime 4–6 ns) afterglow [10–12], arising from emissive recombination of $P870^+$ and I^- [10–14]. The spectrum of this afterglow is the same as that of the prompt fluorescence.

We investigated the effect of energization of *Rhodospirillum rubrum* chromatophores on the parameters of ns recombination luminescence. Generation of $\Delta\Psi$ across the chromatophore membrane was demonstrated to provoke an increase in intensity and shortening of the lifetime of the nanosecond afterglow. This observation enables us to suggest that the primary intermediate step in charge separation between P870 and I is electrogenic.

Abbreviations: CCCP, carbonylcyanide *m*-chlorophenylhydrazone; FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; PP_i, inorganic pyrophosphate; TB^-, tetraphenylborate anions; $\Delta\Psi$ and ΔpH, electrical and chemical components of transmembrane electrochemical gradient of protons; φ and τ, relative quantum yield and lifetime of chromatophore emission

2. Methods

Growing of *Rhodospirillum rubrum* cells (wild-type strain no. 1 MGU), isolation of chromatophores and monitoring of membrane potential generation by the uptake of penetrating tetraphenylborate anions (using a phospholipid membrane as a selective electrode) were done as in [15,16].

The luminescence lifetime and relative quantum yield were measured with a phase-type fluorometer [11].

The incubation mixture contained 220 mM sucrose, 50 mM Tris–HCl buffer (pH 7.6), 2 mM $MgSO_4$, chromatophores ($A^{1\,cm}_{880\,nm}$ amounted to 0.75–1.5 for luminescence measurements and 1.5 for membrane potential measurements). Sodium tetraphenylborate (TB^-) was added at 1×10^{-6} M for experiments in which the TB^- uptake was monitored. Some experiments were done under anaerobic conditions: 0.17 mg/ml glucose oxidase (EC 1.1.3.4), 0.17 mg/ml catalase (EC 1.11.1.6) and 30 mM glucose were added to the incubation mixture; refined sunflower oil was layered onto the aqueous surface.

3. Results

In chromatophores of purple bacteria incubated at a low ambient redox potential the short-lived afterglow, resulting from recombination of $P870^+$ and I^-,

is observed along with the bacteriochlorophyll prompt fluorescence [11]. The total emission is characterized by an increased lifetime (τ) and a decreased quantum yield (φ) as compared to the prompt fluorescence measured under light saturation conditions.

Addition of dithionite to anaerobic *Rhodospirillum rubrum* chromatophores causes increase in τ from 0.25–2.70 ns and 2-fold decrease in φ (fig.1A). Upon subsequent energization of the chromatophore membrane by PP_i τ drops 1.5-fold and φ rises 1.3-fold. The effect of PP_i is removed by a protonophorous uncoupler (fig.1A), suppressed by NaF, an inhibitor of pyrophosphatase, and reversed when the added pyrophosphate is exhausted in the course of hydrolysis catalyzed by chromatophore pyrophosphatase (not shown). Similar changes in τ and φ are observed when the chromatophores are energized by ATP. The ATP effect is removed by uncouplers and by *N,N'*-dicyclohexylcarbodiimide (not shown).

Fig.1B shows that both illumination and addition of PP_i to aerobic chromatophores bring about membrane potential generation monitored by the uptake of penetrating tetraphenylborate anions. These effects are reversed when the light is switched off or the added pyrophosphate is exhausted. In agreement with the data in [17], the light-induced membrane potential generation in the chromatophores incubated anaerobically with dithionite is inhibited, whereas the PP_i-dependent response that is sensitive to uncouplers is not affected considerably.

Antimycin A inhibits the light-induced TB^- response in aerobic chromatophores, but only slightly decreases the level of PP_i-dependent TB^- uptake (fig.2A, [18]). Successive additions of antimycin, PP_i and uncoupler CCCP do not influence the parameters (τ and φ) of the prompt fluorescence of the chromatophores incubated aerobically without dithionite (fig.2B). Hence, the effect of chromatophore energization under reducing conditions may be exclusively attributed to the recombination luminescence.

As shown in [10–14], a transition of reaction centers to the state P870 Q_1^- is necessary for ns luminescence to appear. It can be achieved not only by chemical, but also by photochemical reduction of Q_1 at moderate ambient redox potentials: upon illumination of chromatophores incubated anaerobically with *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD) and ascorbate (fig.3). Addition of PP_i under these conditions leads to a decrease in τ and an increase in φ, as in the presence of dithionite.

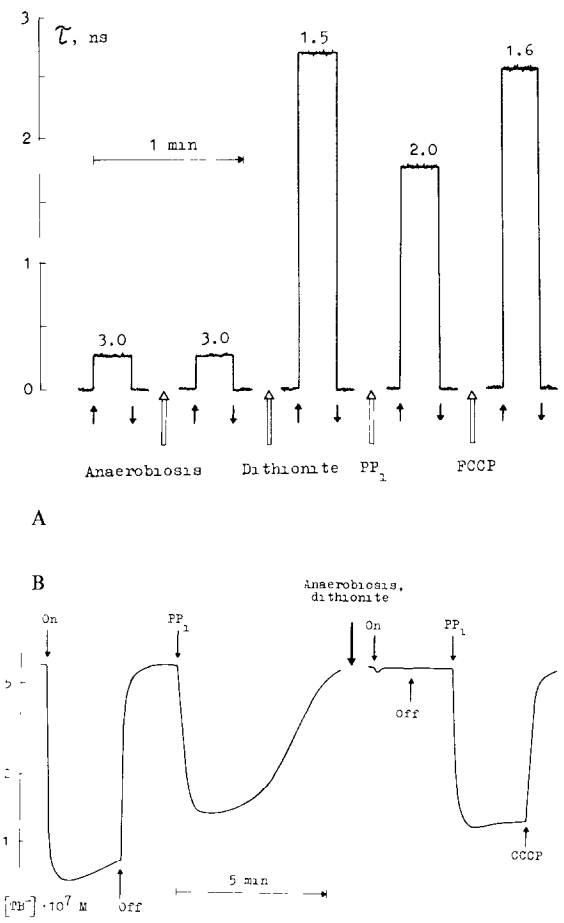

Fig.1. (A) Effect of PP_i and uncoupler FCCP on the lifetime (τ) and relative quantum yield (numbers above the columns) of the total emission of *R. rubrum* chromatophores under reducing conditions. Measurements of τ were made as follows: First, the zero level of τ was determined by placing a latex suspension into an exciting beam and recording a phase-shift of the scattered light as compared to the reference signal. Then, the latex was replaced by a luminescent sample and a phase-shift of the luminescence in relation to the zero level was recorded. The value of this shift was used to calculate τ of the luminescence, according to the formula in [11]. (↑) and (↓) designate the moments when the sample was placed into the light and then removed. Luminescence was excited by 404 and 436 nm lines from a high-pressure mercury lamp and detected through a combination of glass filters cutting off the $\lambda < 760$ nm range. Additions: 1 mg/ml $Na_2S_2O_4$, 0.25 mM PP_i, 1×10^{-6} M FCCP.

Fig.1. (B) Energy-linked uptake of penetrating TB^- by *R. rubrum* chromatophores under aerobic and anaerobic (+ dithionite) conditions. Successive additions: 4×10^{-5} M PP_i; 1 mg $Na_2S_2O_4$/ml; 1×10^{-4} M PP_i; 5×10^{-6} M CCCP. On and off, switching on and off the light ($\lambda > 660$ nm) of saturating intensity.

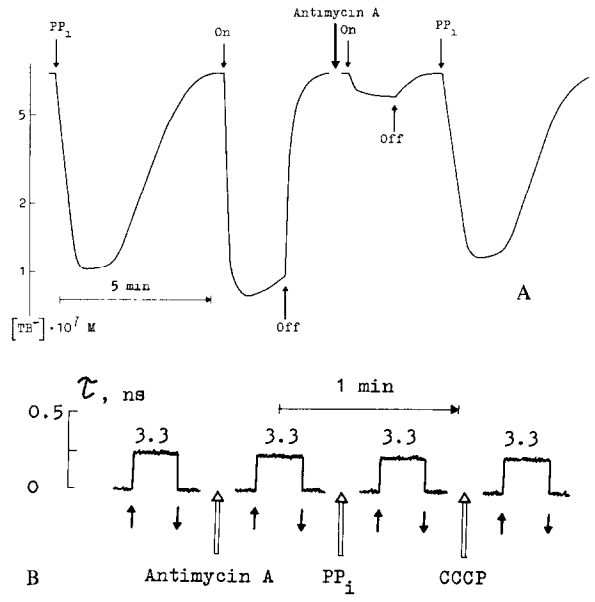

Fig.2. (A) Effect of antimycin A on the light- and PP_i-induced uptake of penetrating TB^- by aerobic *R. rubrum* chromatophores. (B) Effect of antimycin A, PP_i and CCCP on the lifetime (τ) and relative quantum yield (numbers above the columns) of the prompt fluorescence of aerobic *R. rubrum* chromatophores. Additions: 4×10^{-5} M (A) and 1×10^{-4} M (B) PP_i; 1×10^{-6} M antimycin A; 1×10^{-6} M CCCP.

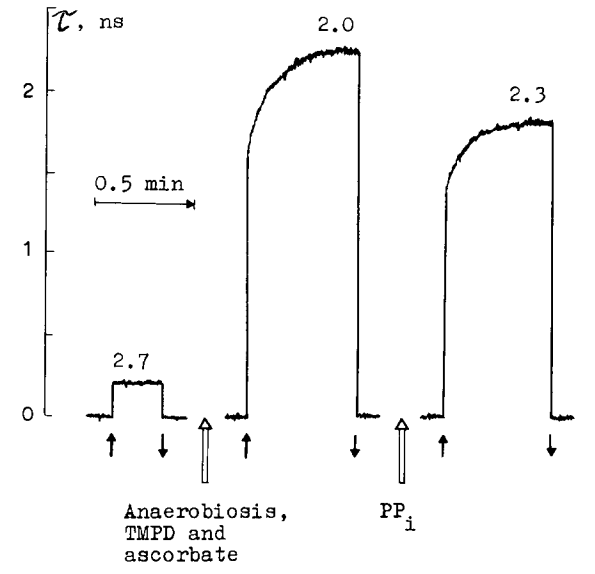

Fig.3. Effect of PP_i on the total emission of *R. rubrum* chromatophores incubated anaerobically with TMPD and ascorbate. Additions: 0.1 mM TMPD; 5 mM sodium ascorbate; 0.1 mM PP_i.

Table 1
Effect of agents, influencing $\Delta\Psi$ and ΔpH, on TB^- uptake, luminescence lifetime (τ) and yield (φ) of *R. rubrum* chromatophores energized by PP_i under anaerobic conditions

Additions	Conc. (M)	TB^- uptake (%)	$\Delta\tau$ (%)	$\Delta\varphi$ (%)
None	–	100	100	100
NaSCN	1×10^{-2}	67	65	
NaSCN	3×10^{-2}	55	50	55
NH_4Cl	2×10^{-2}	121	114	112
Valinomycin[a]	1×10^{-6}	2	0	45
Nigericin + valinomycin[a]	1×10^{-6} 1×10^{-6}	0	0	0

[a] For these experiments the chromatophores were preincubated in 50 mM Tris–HCl buffer containing 75 mM KCl and 2 mM $MgSO_4$ for 2 days, then diluted with the same buffer

The incubation mixture contained 1 mg sodium dithionite/ml and 0.1 mM PP_i. 100% level of TB^- uptake corresponded to the electric potential difference across the measuring phospholipid membrane of 45–56 mV. 100% changes in τ and φ amounted, respectively, to 0.60–0.95 ns and 0.2–0.3 of the luminescence yield measured in the absence of PP_i

In a final series of experiments, we investigated the role played by the electric ($\Delta\Psi$) and chemical (ΔpH) components of transmembrane H^+ electrochemical potential difference in the effect described. As shown in table 1, penetrating SCN^-, by decreasing $\Delta\Psi$ and increasing ΔpH, equally suppress the PP_i-dependent TB^- response and changes in τ and φ. On the contrary, NH_4Cl enhances these changes. Valinomycin eliminates the TB^- response, restores τ to the initial level and diminishes the PP_i effect on φ. The value of φ is restored upon addition of the nigericin + valinomycin combination, acting similarly to protonophorous uncouplers.

Experiments on non-enzymatic $\Delta\Psi$ generation were also done. Chromatophores were placed into an anaerobic medium containing 100 mM KCl and dithionite. Addition of valinomycin under these conditions gave rise to a diffusion potential across the chromatophore membrane, accompanied by a decrease in τ and an increase in φ of the luminescence. The magnitude of these effects relaxing within 1.5–2 min was ~50% of those observed with PP_i.

The data obtained indicate that the bacteriochlorophyll recombination luminescence in the chromatophores essentially depends on the transmembrane difference of electric potentials.

4. Discussion

Recent studies using ps spectroscopy demonstrated that the states, arising from charge separation in bacterial reaction centers with Q_1 prereduced, are identical with those occurring when Q_1 is oxidized [8]. On this basis, one may believe that the state, the radiative decay of which manifests in ns recombination luminescence, is an intermediate in photosynthetic charge separation and its energy characteristics reflect the actual thermodynamic parameters of the reaction centers.

Assuming the effect of membrane energization on the luminescence parameters to be entirely due to the membrane potential action on activation energy of the recombination luminescence, we concluded that in our experiments $\Delta\Psi$ generation across the chromatophore membrane caused shortening of the recombination luminescence lifetime from 4–6 ns to 2–3 ns and a 1.5–2-fold increase in its yield. According to the kinetic description of the photosynthetic primary processes in [11], this may take place when the luminescence activation energy decreases ~2-fold: from 50–70 meV to ~30 meV.

Thus, the results obtained point out that the primary intermediate step of charge separation in the reaction centers of photosynthetic bacteria may be electrogenic.

References

[1] Steiner, L. A., Lopes, A. D., Okamura, M. Y., Ackerson, L. G. and Feher, G. (1974) Fed. Proc. FASEB 33, 1461, abstr. 1345.
[2] Reed, D. W., Raveed, D. and Reporter, M. (1975) Biochim. Biophys. Acta 387, 368–378.
[3] Drachev, L. A., Kondrashin, A. A., Samuilov, V. D. and Skulachev, V. P. (1975) FEBS Lett. 50, 219–222.
[4] Drachev, L. A., Frolov, V. N., Kaulen, A. D., Kondrashin, A. A., Simuilov, V. D., Semenov, A. Yu. and Skulachev, V. P. (1976) Biochim. Biophys. Acta 440, 637–660.
[5] Valkirs, G., Rosen, D , Tokuyasu, K. T. and Feher, G. (1976) Biophys J. 16, 223a, abstr. F-PM-D9.
[6] Zurrer, H., Snozzi, M., Hanselmann, K. and Bachofen, R. (1977) Biochim. Biophys. Acta 460, 273–279.
[7] Oelze, J. (1978) Biochim. Biophys. Acta 509, 450–461.
[8] Dutton, P. L., Prince, R. C., Tiede, D. M., Petty, K. M., Kaufmann, K. J., Netzel, T. L. and Rentzepis, P. M. (1977) in: Chlorophyll–proteins, reaction centers and photosynthetic membranes (Olson, J. M. and Hind, G. eds) pp. 213–237, Upton, New York.
[9] Fleischman, D. E. (1978) in: The photosynthetic bacteria (Clayton, R. K. and Sistrom, W. R. eds) pp. 513–523, Plenum, New York.
[10] Godik, V. I. and Borisov, A. Yu. (1977) FEBS Lett. 82, 355–359.
[11] Godik, V. I. and Borisov, A. Yu. (1979) Biochim. Biophys. Acta 548, 296–308.
[12] Godik, V. I., Kotova, E. A. and Borisov, A. Yu. (1979) Dokl. Akad. Nauk 249, 990–993.
[13] Shuvalov, V. A. and Klimov, V. V. (1976) Biochim. Biophys. Acta 440, 587–599.
[14] Grondelle, R., Holmes, N. G., Rademaker, H. and Duysens, L. N. M. (1978) Biochim. Biophys. Acta 503, 10–25.
[15] Drachev, L. A., Kaulen, A. D., Samuilov, V. D., Severina, I. I., Semenov, A. Yu., Skulachev, V. P. and Chekulaeva, L. N. (1979) Biofizika 24, 1043–1047.
[16] Isaev, P. I., Liberman, E. A., Samuilov, V. D., Skulachev, V. P. and Tsofina, L. M. (1970) Biochim. Biophys. Acta 216, 22–29.
[17] Barsky, E. L. and Samuilov, V. D. (1973) Biochim. Biophys. Acta 325, 454–462.
[18] Remennikov, V. G. and Samuilov, V. D. (1979) Biochim. Biophys. Acta 548, 216–223.