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A STUDY ON THE MEMBRANE POTENTIAL AND pH GRADIENT IN CHROMATOPHORES AND INTACT CELLS OF PHOTOSYNTHETIC BACTERIA

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SUMMARY

Generation of membrane potential ($\Delta\psi$) and transmembrane pH difference (ΔpH) was studied in PP_i-energized chromatophores of *Rhodospirillum rubrum* by means of measurements of carotenoid and bacteriochlorophyll absorption changes, atebrin and 8-anilinonaphthalene-1-sulphonate fluorescence responses, and phenyldicarbaundecaborane transport.

The data obtained are consistent with the suggestion that carotenoid, bacteriochlorophyll and phenyldicarbaundecaborane responses are indicators of $\Delta\psi$, while an atebrin response is an indicator of ΔpH. The fluorescence of 8-anilinonaphthalene-1-sulphonate is affected both by $\Delta\psi$ and ΔpH.

INTRODUCTION

The synthetic penetrating anion PCB^- and the absorption band shifts of carotenoids and bacteriochlorophyll were used as probes for energized state of chromatophores of photosynthetic bacteria [1–6]. Adequacy of the PCB^- method for measurements of membrane potential was directly confirmed by experiments with proteoliposomes [7] and planar phospholipid membranes [8], containing bacteriorhodopsin. There are also other indicators of the energized state of coupling membranes, such as the fluorescence changes of ANS^- and atebrin. It was previously shown that the uptake of ANS^- anions by energized submitochondrial particles (the inner phase has a positive charge) is followed by a dye fluorescence enhancement due to binding of ANS^- by the membrane [9]. A similar response is observed upon illumination of *Rhodospirillum rubrum* chromatophores incubated with ANS^- [10, 11]. The energization of mitochondria (the inner phase has a negative charge) causes the efflux of ANS^- from

Abbreviations: ANS^-, 8-anilinonaphthalene-1-sulfonate; CCCP, 2,4,6-trichlorocarbonyl cyanide phenylhydrazone; PCB^-, phenyldicarbaundecaborane; PP_i, inorganic pyrophosphate.

hydrophobic phase of the membrane and is accompanied by dye fluorescence quenching [9]. On the basis of these data, the conclusion was made that transport of ANS^- across coupling membranes proceeds by an electrophoretic mechanism.

In addition to the electric component ($\Delta\psi$), the electrochemical potential of H^+ ($\Delta\mu_{H^+}$) involves the osmotic (concentration) component given as the transmembrane difference of H^+ concentration (ΔpH) [12]. One of the ΔpH indicators is atebrin fluorescence quenching associated wtih proton uptake in chloroplast or chromatophore suspensions [11, 13].

The purpose of the present work was a further study of energy-dependent responses of ANS^- and atebrin fluorescence and other indicators of energized state in *R. rubrum* chromatophores. Comparison has also been made of fluorescence responses of the dyes in chromatophores and intact cells of photosynthetic bacteria.

METHODS

R. rubrum and *Ectothiorhodospira shaposhnikovii* were grown anaerobically in the light [6]. Cells from 3–5-day-old cultures were washed twice in 0.05 M Tris–HCl buffer (pH 7.6) and used in experiments. The chromatophores of *R. rubrum* were isolated by an ultrasonic treatment [1]. The concentration of bacteriochlorophyll was determined spectrophotometrically. The extinction coefficient was 140 $mM^{-1} \cdot cm^{-1}$ at 880 nm for *R. rubrum* [14]. The carotenoid absorption changes at 570 nm and bacteriochlorophyll changes at 795 nm were measured with a two-wavelength spectrophotometer, Hitachi-356 [5]. The PCB^- concentration changes were monitored by the phospholipid membrane technique [1]. ANS^- (Mg- or NH_4-salts) and atebrin fluorescence were measured using a fluorimeter. The exciting beam was emitted by a cadmium lamp at 327, 340, 346 and 361 nm. Fluorescence was measured from the side of the exciting beam. The saturating intensity of actinic light with λ more than 700 nm was obtained from a tungsten lamp. The atebrin fluorescence life-times were measured with a phase-type fluorometer [15]. Experiments were performed with aerobic suspensions of chromatophores and bacterial cells.

RESULTS AND DISCUSSION

It is known that the electrochemical potential of H^+ in *R. rubrum* chromatophores is generated upon light-induced or dark electron transfer, as well as hydrolysis of ATP or PP_i [1].

Addition of PP_i to suspension of chromatophores (Fig. 1, left) causes (1) an enhancement of ANS^- fluorescence, (2) a quenching in atebrin fluorescence, (3) an uptake of penetrating PCB^- anions by chromatophores, (4) absorption changes of carotenoids and (5) those of bacteriochlorophyll. It was shown that the responses of PCB^-, carotenoids and bacteriochlorophyll are due to electric component ($\Delta\psi$) of the H^+ transmembrane electrochemical potential [1–3]. Fig. 1 (right) shows the results of measurement of the same parameters in an incubation mixture supplemented with nigericin which specifically abolishes the ΔpH component of electrochemical H^+ potential [16]. It is seen that nigericin prevents the atebrin fluorescence quenching and partially depresses ANS^- fluorescence change, while responses of PCB^-, carotenoids or bacteriochlorophyll hardly change at all.

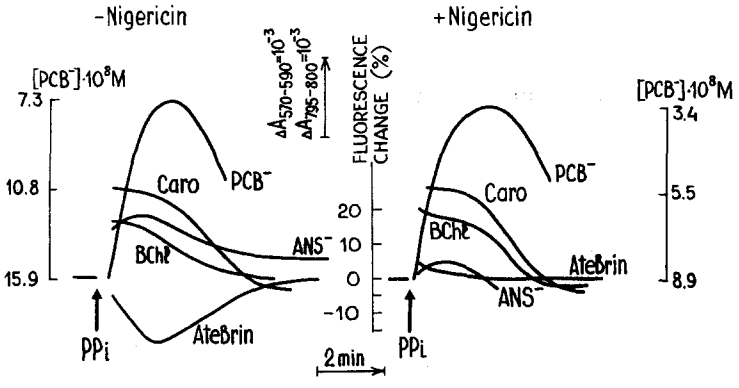

Fig. 1. The effect of the PP_i-induced energization on absorption of bacteriochlorophyll (BChl) at 795 nm and carotenoids (Caro) at 570 nm, the PCB^- concentration and the ANS^- and atebrin fluorescence in suspension of *R. rubrum* chromatophores. Incubation mixture: chromatophores (4 μm bacteriochlorophyll), 0.05 M Tris · HCl buffer (pH 7.7), 0.25 M sucrose, 5 mM $MgCl_2$, 20 mM KCl, and where indicated, 0.1 mM ANS^-, 2.5 μM atebrin, 1.5 μg/ml nigericin. Addition: 25 μM PP_i.

Unlike nigericin, the penetrating SCN^- anions causing the decrease of $\Delta\psi$ and the increase of ΔpH [16], promote the atebrin fluorescence response and depress the absorption changes of carotenoids in chromatophores energized by PP_i (Figs 2 and 3). Moreover, SCN^- decreases the rate of ANS^- response. SCN^- in combination with nigericin and KCl inhibits completely the energization-induced ANS^- fluorescence change (Fig. 4).

The data obtained are in agreement with the suggestion that responses of PCB^-, carotenoids and bacteriochlorophyll may serve as probes for $\Delta\psi$ [1-6] and the atebrin response as a probe for ΔpH [11, 13]. As to the ANS^- responce, it is affected by both $\Delta\psi$ and ΔpH. The effect of the first component seems to be caused by electrophoretic

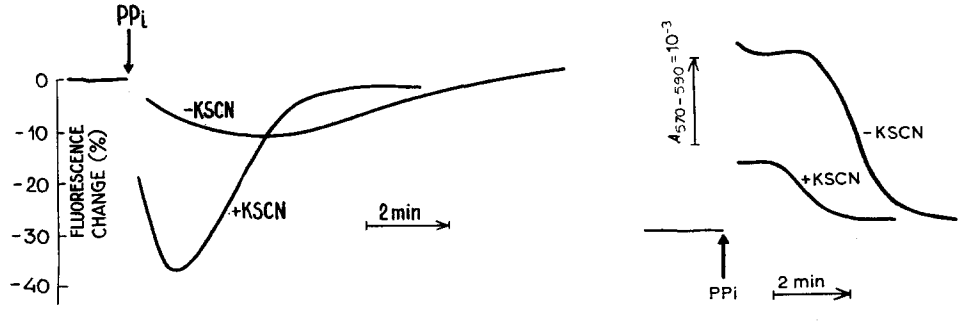

Fig. 2. The effect of thiocyanate on the PP_i-induced changes of atebrin fluorescence in a suspension of *R. rubrum* chromatophores. Incubation mixture: chromatophores (8.5 μM bacteriochlorophyll), 0.05 M Tris · HCl buffer (pH 7.6), 0.25 M sucrose, 5 μM $MgCl_2$, 5 μM atebrin and, where indicated, 10 mM KSCN. Addition: 25 μM PP_i.

Fig. 3. The effect of thiocyanate on the PP_i-induced changes in carotenoid absorption at 570 nm in *R. rubrum* chromatophores. Incubation mixture: chromatophores (14 μM bacteriochlorophyll), 0.05 M Tris · HCl buffer (pH 7.7), 0.25 M sucrose, 5 mM $MgCl_2$ and, where indicated, 30 mM KSCN. Addition: 75 μM PP_i.

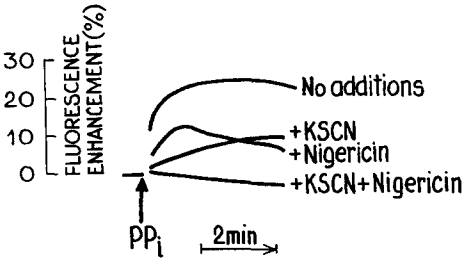

Fig. 4. The effect of nigericin and thiocyanate on the PP_i-induced changes of ANS^- fluorescence in suspension of *R. rubrum* chromatophores. Incubation mixture: chromatophores (9.2 μM bacteriochlorophyll), 0.05 M Tris · HCl buffer (pH 7.5), 0.25 M sucrose, 5 mM $MgCl_2$, 20 mM KCl, 80 μM ANS^-. Additions: 1.5 μg/ml nigericin, 10 mM KSCN, 50 μM PP_i.

transmembrane movement of penetrating ANS^- into chromatophores, i.e. from the incubation mixture into the positively charged chromatophore interior, occupying an insignificant part of the total volume of the test sample. As a consequence of these differences in the size of the intra- and extrachromatophore volumes, the increase of ANS^- concentration inside chromatophores must be larger than the decrease of ANS^- concentration outside. Correspondingly, accumulation of ANS^- inside chromatophores should increase both the membrane-bound ANS^- and its fluorescence [9, 17]. Such an explanation is in accord with the fact of the ANS^- emission enhancement caused by a diffusion potential of K^+ (+ valinomycin) or H^+ (+ protonophore) in mitochondria and submitochondrial particles ("plus" inside) [9, 18, 19].

The inequality of intra- and extrachromatophore volumes can also produce an ΔpH effect. The acidification of intrachromatophore solution upon energization must be larger than the alkalinization on the outside of chromatophores. An increase of protonated positively charged groups on the inner surface of chromatophore membrane upon acidification of the chromatophore interior must intensify the binding of ANS^- anions and, correspondingly, enhance its fluorescence. This suggestion is supported by the data in Fig. 5. It is seen that the acidification of incubation mixture with

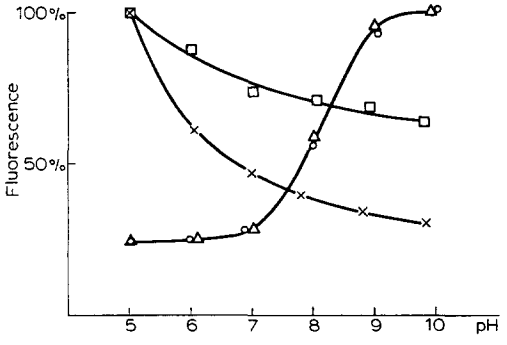

Fig. 5. Dependence of ANS^- and atebrin fluorescence on pH value. Incubation mixture: 0.05 M Tris/acetate buffer, 80 μM ANS^- or 5 μM atebrin, and where indicated chromatophores of *R. rubrum* (6 μM bacteriochlorophyll), □—□ ANS^-; ×—×, ANS^- + chromatophores; ○—○, atebrin; △—△, atebrin + chromatophores.

nonenergized chromatophores causes an enhancement of ANS^- emission. This ANS^- response is not affected by the uncoupler CCCP. In the absence of chromatophores, ANS^- fluorescence is slightly affected by pH.

It is noteworthy that the effects of $\Delta\psi$ and ΔpH upon the ANS^- fluorescence are of the same direction. The appearance of an excess of positive charges inside chromatophores is accompanied by acidification of intrachromatophore solution. Both of these changes were found to increase ANS^- fluorescence.

The above explanation of the mechanism of ΔpH action on the ANS^- fluorescence can also be applied to the response of atebrin. This dye represents a weak base. The acidification of intrachromatophore solution causes movement of the deprotonated atebrin into chromatophores down the pH gradient. Since the deprotonated form of atebrin is uncharged, the electric component of $\Delta\bar{\mu}_{H^+}$ has no influence on the movement of this compound in the membrane. Several explanations have been advanced to describe the mechanism of the energy-dependent quenching of atebrin fluorescence [20, 21]. Our experiments with an aqueous solution of atebrin showed that there are at least two factors inducing the atebrin fluorescence quenching in energized chromatophores, namely, atebrin protonation and stacking. The dependence of the atebrin fluorescence level on pH is shown in Fig. 5. Dye fluorescence decreased significantly as pH decreased from 10 to 7. The fluorescence level is not affected within the pH range 7–5. Similar data were obtained by Deamer et al. [21]. Addition of chromatophores to atebrin solution has no influence on fluorescence quenching linked with pH change. The dependence of atebrin fluorescence on pH indicates that the protonation of dye in the intrachromatophore solution can induce a fluorescence decrease.

The increase in the intrachromatophore atebrin concentration may be another factor causing fluorescence quenching. It was shown [13] that the transmembrane concentration gradient of atebrin in chloroplasts can be as high as 10 000.

The concentration dependence of the atebrin fluorescence level is illustrated in Fig. 6. Maximal fluorescence level is observed at a concentration of about 0.25 mM. The fluorescence of the dye is completely quenched at a concentration of 50 mM.

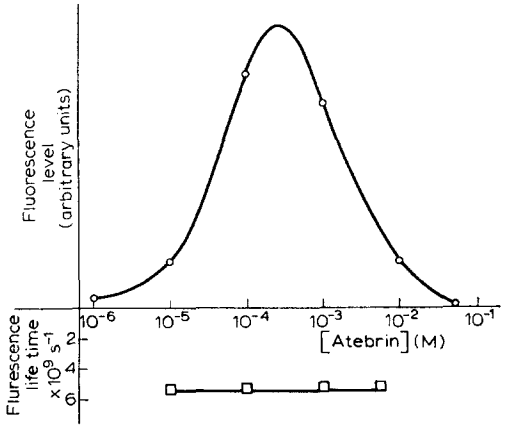

Fig. 6. Concentration dependence of fluorescence level (○—○), and fluorescence life-time (□—□), of atebrin in the 50 mM potassium phosphate buffer (pH 7.8).

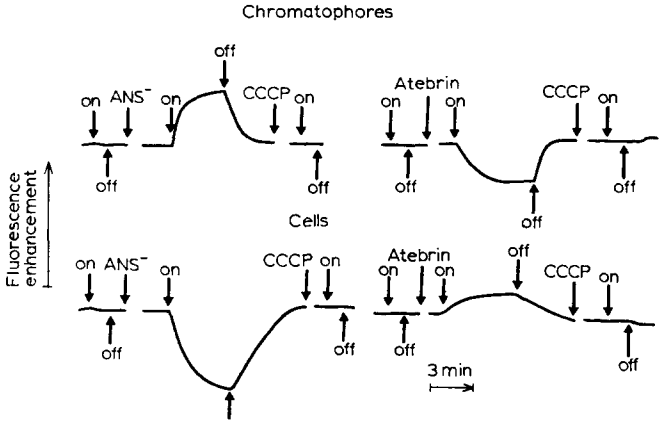

Fig. 7. The light-induced changes of ANS^- and atebrin fluorescence in suspensions of *R. rubrum* chromatophores and intact cells. Incubation mixture: cells or chromatophores (50 μM bacteriochlorophyll), 0.05 M Tris · HCl buffer (pH 7.8), 0.25 M sucrose. Additions: 0.1 mM ANS^-, 0.5 μM atebrin, 10 μM CCCP. On, light on; Off, light off.

The atebrin fluorescence life-time was measured in order to elucidate the nature of concentration quenching. The life-time was found to be equal to 5.2–5.4 ns and was not concentration dependent (Fig. 6). Thus, the concentration quenching of atebrin fluorescence belongs to the second type of quenching [22], which is connected with the formation of non-fluorescent dye complexes. A stacking interaction of atebrin molecules seems to be most probable. As experiments showed, addition of glycerol increases the fluorescence level at high atebrin concentrations, the effect suggesting stacking complex decomposition.

A comparison of light-induced responses of ANS^- and atebrin fluorescence was made in further experiments with cells and chromatophores of *R.rubrum* (Fig. 7). It is seen that illumination of chromatophores causes an enhancement of ANS^- fluorescence similar to that induced by PP_i. Atebrin fluorescence is quenched upon illumination of chromatophores. The direction of light-induced changes of ANS^- and atebrin fluorescence in a suspension of *R. rubrum* cells is opposite to that in a chroma-

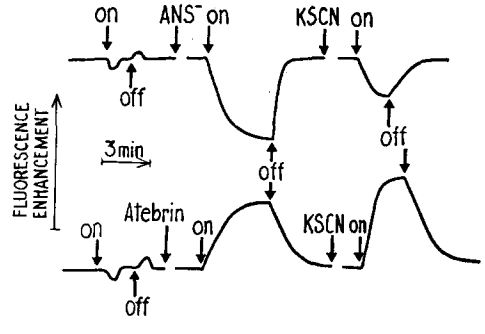

Fig. 8. The effect of thiocyanate on the light-induced changes of ANS^- and atebrin fluorescence in suspension of *E. shaposhnikovii* cells. $A_{594\ nm} = 0.75$. KSCN concentration was 5 mM. Other conditions as in Fig. 7.

tophore suspension. The responses of ANS^- and atebrin fluorescence in suspensions of cells or chromatophores are reversed in the dark and prevented by the uncoupler CCCP.

Similar data were obtained in the cells of the purple sulphur bacteria *E. shaposhnikovii* (Fig. 8). Small fluorescence changes in illuminated cell suspension observed in the absence of ANS^- or atebrin are probably connected with the reduction of intracellular nicotinamide dinucleotides. Upon subsequent addition of fluorochromes the illumination causes the quenching of ANS^- fluorescence and enhancement of atebrin fluorescence. Thiocyanate depresses the ANS^- response and promotes the atebrin response.

Thus, cells and chromatophores of photosynthetic bacteria demonstrate ANS^- and atebrin fluorescence responses of opposite direction. As was shown earlier, similar relationships are inherent in the responses of penetrating anions PCB^- and tetraphenylboron as well as of the triphenylmethylphosphonium cation [23, 24]. The data obtained are in accordance with the assumptions that the inner phase of cytoplasmic membrane has a negative charge whereas the inner phase of chromatophores is positively charged [23–26].

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