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LIGHT- AND DIFFUSION-POTENTIAL-INDUCED SHIFT OF CAROTENOID SPECTRUM IN RECONSTITUTED VESICLES OF *RHODOPSEUDOMONAS SPHAEROIDES*

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SUMMARY

Proteoliposomes were reconstituted from detergent-solubilized pigment · protein complexes of chromatophores of *Rhodopseudomonas sphaeroides* and soybean phospholipids. The reconstituted vesicles showed a photooxidation of reaction center bacteriochlorophyll and a light-induced spectral shift of carotenoid to longer wavelengths. The red shift similar to that in intact cells or chromatophores, indicates the generation of local fields in the membrane of proteoliposomes. When inside-positive membrane potential was induced by adding valinomycin and potassium salt, a shift of carotenoid spectrum to shorter wavelengths was observed. Therefore, the reconstituted vesicles, at least in the major part of population, produced the light-induced local field in the same direction as in intact cells, which is inside negative. Sidedness of the membrane structure and the direction of electric field formation in reconstituted vesicles were opposite to those in chromatophores (inside-out vesicles).

INTRODUCTION

Sidedness and asymmetry of molecular organization of energy-transducing membranes are closely correlated to their function which involves the generation and utilization of transmembrane electrochemical potential gradient of H^+ [1]. In photosynthetic membranes of bacteria, localizations of cytochrome c_2 [2, 3] and reaction center complex [4, 5] have been studied and their relation to energy conversion discussed.

Methods for reconstituting membrane vesicles with activities involved in energy conversion have been elaborated and applied to the analyses of oxidative phosphorylation in mitochondria [6, 7] and bacteria [8], and photosynthesis in bacteria and chloroplasts [9–11]. In those systems, the conversion of energy of ATP [6–8] or light [9–11] into electrochemical potential energy has been demonstrated by the fluorescence probe method or the combination of a planar membrane and electrodes. An asymmetrical reconstitution of membranes was demonstrated by the latter method.

In certain photosynthetic bacteria, the electrochromic spectral shift of carotenoid has been shown to indicate membrane potential generated by light or concentration gradient of ions [12, 13]. The carotenoid shift was also used to study the molecular orientation in different membrane structures when the shift was induced by diffusion potential [14]. The diffusion-potential-induced carotenoid shift in the proteoliposome vesicles reconstituted from the pigment · protein complexes of *Rhodopseudomonas sphaeroides* was analyzed in the present study.

MATERIALS AND METHODS

Cultures of *R. sphaeroides*, a species of non-sulphur purple bacteria, were grown under illumination in a medium containing 0.2 % casamino acids, 0.3 % yeast extract, 0.8 mM $MgSO_4$, 0.2 mM $CaCl_2$, and 0.02 mM $FeSO_4$. Chromatophores were prepared by differential centrifugation (between 20 000 × *g*, 30 min and 100 000 × *g*, 1.5 h) after the French-press disruption of the cells at 1000 kg/cm^2 in 5 mM EDTA/5 mM sodium phosphate (pH 7.4), and washed with the same buffer.

Chromatophores were solubilized by suspending them in a mixture of 1 % sodium dodecyl sulfate and 5 mM sodium phosphate (pH 7.4) at room temperature. The density of chromatophores was adjusted to give an absorbance of approx. 30 cm^{-1} at 850 nm. The supernatant obtained by ultracentrifugation (100 000 × *g*, 1.5 h) which contained most of the pigments, was applied to a column of Sephadex G-200 gel (1.8 cm in diameter and 68 cm long) equilibrated with 1 % sodium cholate and 5 mM sodium phosphate (pH 7.4), and eluted with the same buffer. A fraction with high contents of bacteriochlorophyll (monitored at 850 and 800 nm), carotenoids (507 nm) and proteins (280 nm) was eluted in a single band as the void-volume fraction. The fraction showed a spectrum (250–900 nm) similar to that of the chromatophores, except that (a) the shoulder of bacteriochlorophyll at 870 nm was not distinct, (b) the bacteriochlorophyll band at 800 nm and the carotenoid bands were slightly (2–5 nm) blue-shifted, and the former was broadened and (c) the absorbance ratio A_{280}/A_{850}, decreased from 0.8 to 0.5 (after the correction for the scattering effect). Cytochrome c_2 was not detected in the fraction and the cytochrome *b*/bacteriochlorophyll ratio was similar to chromatophores judged from the dithionite-reduced-minus-oxidized difference spectrum with a peak at 562 nm.

Proteoliposomes were reconstituted by a method similar to that described by Drachev et al. [9, 10] from the gel-filtered fraction and phospholipids partially purified [6] from soybean "lecithin" (Tokyo Kasei). The mixture containing 50 mg of soybean phospholipids and the pigment · protein complex (150 nmol bacteriochlorophyll) in 3.5 ml of 2 % sodium cholate, 5 mM $MgSO_4$, and 50 mM sodium phosphate (pH 7.4), was dialyzed against 0.5 mM dithiothreitol/50 mM sodium phosphate (pH 7.4). After collecting by ultracentrifugation (100 000 × *g*, 1.5 h), the reconstituted proteoliposomes were resuspended in a mixture of 0.25 M sucrose/2 mM $MgSO_4$/5 mM sodium phosphate (pH 7.4). Bacteriochlorophyll was determined, without extraction, using the absorption coefficient given by Clayton (ε_{mM} = 95 at 850 nm) [15]. Measurements of the light-induced and the diffusion-potential-induced absorbance changes were performed as described previously [14].

RESULTS AND DISCUSSION

The time courses of the absorbance changes of carotenoids, induced by light and diffusion potential in the reconstituted vesicles, are shown in Fig. 1. The spectra of the changes are depicted in Fig. 2. When light was turned on, an absorbance change took place in the spectral region of carotenoids, as in intact cells or chromatophores [12, 14]. The light-minus-dark difference spectrum corresponding to the 507 nm band, showed a positive maximum at 518 nm and a negative maximum at 501 nm. The difference spectrum was shifted to blue in about 6 nm as compared with that in intact cells, spheroplasts or chromatophores [12, 14]. It was in good agreement with the first derivative of the absorption spectrum of the liposome preparation (signs inverted) (Fig. 2, dashed line). These data indicate that the light-induced change in

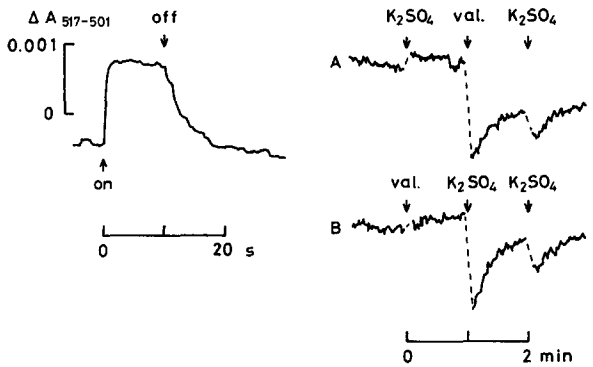

Fig. 1. Absorbance changes of carotenoid induced by light and K^+ gradients in the reconstituted vesicles. The reaction mixture contained the vesicles (equivalent to 12 μM bacteriochlorophyll), 0.25 M sucrose, 2 mM $MgSO_4$ and 5 mM sodium phosphate (pH 7.4). Time course on the left side is the light-induced absorbance change. Changes in absorbance caused by additions of valinomycin and K_2SO_4 are shown in the traces on the right with different orders of additions (A and B). Valinomycin added was 130 nM, K_2SO_4 additions were 1.8 mM each.

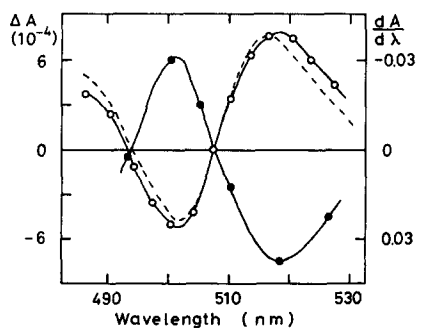

Fig. 2. Absorption spectrum changes caused by light and K^+ gradient in the reconstituted vesicles. Light-induced changes (○—○) and valinomycin-induced changes after 1.8 mM K_2SO_4 addition (●—●) were measured in a series of experiments similar to those in Fig. 1. Changes which occurred 5 s after the start of illumination, or those which occurred 5 s after the addition of valinomycin are plotted. Reference wavelength, 507 nm. Dashed line represents the first derivative of the absorption spectrum of the sample.

reconstituted vesicles is also a red shift of the spectrum of carotenoid. It may be noted that the first derivative of the absorption spectrum and the light-induced difference spectrum were not identical in chromatophores [16, 17, 24]. It remains to be clarified whether a similar variance can be also observed in the reconstituted vesicles or not.

An addition of K_2SO_4 in the absence of valinomycin or that of valinomycin in the absence of K^+ induced no large changes in the difference absorbance (517 minus 501 nm). However, when both valinomycin and potassium salt were added, a decrease in the difference absorbance, corresponding to the blue shift of the carotenoid spectrum, took place at this wavelength pair, regardless of the order of additions (Figs. 1 and 2). The valinomycin-K^+-induced difference spectrum had a positive maximum at 501 nm and a negative maximum at 518 nm. These results indicate that the spectral change of carotenoid, probably an electrochromic band shift, is induced by the membrane potential in the proteoliposomes as in spheroplasts, spheroplast membrane vesicles and chromatophores.

Fig. 3 shows the change in difference absorbance (517 minus 501 nm) induced by valinomycin in the presence of K^+ of various concentrations (cf. trace A, Fig. 1). A linear relationship between the absorbance change and the logarithm of concentration of K^+ was observed. Theoretically, the diffusion potential is linearly related to log $[K^+]_{out}$ when $[K^+]_{in}$ is constant. The data in Fig. 3 indicate that the blue-shift type spectral change is induced by the inside-positive membrane potential. A similar diffusion potential dependence has been observed in spheroplasts and spheroplast membrane vesicles [14], and in chromatophores (with signs inverted) [12, 14].

The detergent-solubilized membrane, the gel-filtrated pigment · protein complex, or the dialysate without phospholipids did not show the typical spectral change induced by illumination or diffusion potential, observed in intact cells or vesicular membrane preparations, as well as in the reconstituted vesicles. However, a small light-induced absorption spectrum change in the carotenoid region was observed in these non-vesicular preparations. The spectral shape was different from that of the typical shift-type change (data not shown). This is consistent with the idea that most of the electrochromic spectral change is not expected without vesicular structures (or structures which maintain the electrochemical gradient).

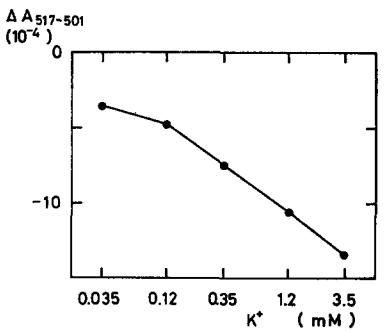

Fig. 3. Dependence of valinomycin-induced carotenoid change on concentration of previously added K^+. The changes were measured as in trace A of Fig. 1 with various concentrations of K_2SO_4. Values observed 5 s after the valinomycin addition are plotted.

In the membrane vesicles prepared from this bacterial species, when inside-positive membrane potential was applied by valinomycin and potassium salt the 'blue shift' was observed in spheroplasts and spheroplast membrane vesicles which conserved the membrane orientation of intact cells. On the other hand, the 'red shift' took place in chromatophores (inside-out vesicles) [14]. Therefore, it is concluded that the reconstituted proteoliposomes have the same orientation of potential-sensing carotenoid molecules or carotenoid complexes as intact cells. The light-induced change, which was of the 'red shift' type, indicates the generation of an outside-positive local field within the membrane. Therefore, the direction of membrane-potential generation by light in the proteoliposomes is also the same as that in cells. However, the data do not exclude the possibility of reconstituted vesicles with less organized molecular arrangement or a part of the vesicles having the random or opposite orientation. As Drachev et al. have suggested for the reconstituted vesicles from *Rhodospirillum rubrum* [10], the orientation of protein or pigment molecules in proteoliposomes may be regulated by the characteristics and composition of molecules constituting the liposome vesicles, and by pH of the medium used [18].

Jackson and Crofts [13] and Jackson and Dutton [19] have resolved the flash-illumination-induced carotenoid shift into three phases. Phase I (about a third or a quarter of the whole change) was interpreted to correspond to the charge separation at the reaction center. In the reconstituted vesicles photooxidation of the reaction center bacteriochlorophyll was also observed with an absorbance decrease at 605 nm [20]. Illumination induced an absorbance decrease of 0.0023 at 605 nm with a reference wavelength of 575 nm in a sample containing 20 μM bacteriochlorophyll. The absorbance decrease was similar in extent to that of chromatophores. On the other hand, no photooxidation of cytochrome c_2 could be detected. The electric field formed within the membrane of vesicles reconstituted by the charge separation and the succeeding processes probably induce the electrochromic spectral shift of carotenoid. The size of the carotenoid shift was about a tenth of that in chromatophores. The decreased magnitude may be ascribed to a partially random distribution of the membrane components and/or irreversible damage(s) caused by detergents.

The carotenoids in the membrane of the photosynthetic bacteria have at least two types of binding or molecular associations. In one type, carotenoid molecules are closely associated with the reaction center [21], and in another, with the bulk pigment system. Both in the bulk pigment system and in the reaction center complex, carotenoid molecules are probably tilted out of the membrane plane [21–23]. The relationship between the carotenoid shift and the nature of molecular associations may be clarified using reconstituted systems.

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