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RECONSTITUTION OF ELECTROGENIC FUNCTION IN ISOLATED PIGMENT-PROTEIN COMPLEXES OF *ANABAENA VARIABILIS*

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Treatment of *Anabaena variabilis* membranes with lauryldimethylamine *N*-oxide yielded two fractions of pigment-protein complexes which were separable by gel filtration on Sepharose 6B. A green fraction was characterized which had a maximum of the chlorophyll long-wave absorption band at 678 nm and a small amount of carotenoid. In this fraction, Photosystem I activity was higher than in another (brownish-green) fraction which had a maximum of the chlorophyll absorption band at 673 nm and which was enriched in carotenoids. Similarly to isolated membranes, proteoliposomes containing pigment-protein complexes took up tetraphenylborate anions and tetraphenylphosphonium cations and were found to be capable of light-dependent membrane potential generation, when associated with a planar phospholipid membrane in the presence of reduced phenazine methosulfate upon illumination. The spatial arrangement of the pigment-protein complexes in the native and artificial membranes is discussed.

Introduction

According to the chemiosmotic concept [1], an H^+ electrochemical potential difference including the electrical ($\Delta\psi$) and chemical (ΔpH) components is generated across energy-transducing biological membranes. The magnitude of ΔpH in thylakoids (alkaline outside) of chloroplasts [2–4] and cyanobacteria [5–7] amounts to 2–3 pH units. Light-induced generation of ΔpH is also observed in proteoliposomes containing isolated PS I reaction center complexes from pea chloroplasts [8]. The proteoliposomes appear to include two fractions, one of which causes acidification and the other alkalinization of the incubation medium in the light.

The steady-state magnitude of $\Delta\psi$ in illuminated chloroplasts (with a positive charge inside thylakoids) does not exceed 10–15 mV [2]. The $\Delta\psi$ is also generated by PS I complexes from pea chloroplasts reconstituted into phospholipid vesicles [9]. Under conditions of flash excitation, the magnitude of $\Delta\psi$ in thylakoids was about 50 mV [10]. The electrical potential difference across the cytoplasmic membrane (with a negative charge inside) equaled 75–80 mV in illuminated cells of the cyanobacterium *Anabaena variabilis* [11,12].

The data presented in this paper indicate that the photoactive pigment-protein complexes from *A. variabilis* reconstituted into phospholipid vesicles generate a transmembrane electrical potential difference upon illumination.

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; TPP^+, tetraphenylphosphonium cation; TPB^-, tetraphenylborate anion; PS, photosystem; Chl, chlorophyll.

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Methods

Cells of *A. variabilis* Kütz. No. 458 from the Algae Culture Collection of Leningrad University were grown phototrophically in medum 'C' [13]. The cells were washed twice with 50 mM sodium phosphate buffer (pH 7.0), suspended in the same buffer (absorbance of the cell suspensions was 60–80 units at 680 nm) and sonicated six times for 10 s at 30-s intervals in an ice bath using an ultrasonic disintegrator UZDN-1 (0.4 mA, 22 kHz). The homogenates obtained were subjected to differential centrifugation at $8000 \times g$ for 10 min and $144000 \times g$ for 1 h at 0–4°C. The photosynthetic membranes sedimented were washed with the same buffer.

The membrane fraction was suspended in 50 mM sodium phosphate buffer (pH 7.0) to a final absorbance of 40 units at 680 nm. 0.25% lauryldimethylamine *N*-oxide was added after which the suspension was stirred for 1 h at 0–4°C and centrifuged for 1.5 h at $144000 \times g$. The supernatant containing pigment-protein complexes (absorbance was 20–25 units at 676 nm) was subjected to gel filtration: 3.5 ml of the suspension were passed through a Sepharose 6B (2×45 cm) column equilibrated with 50 mM sodium phosphate buffer (pH 7.4) containing 0.025% lauryldimethylamine *N*-oxide. The complexes were eluted with the same solution and collected in 1.5-ml fractions in the dark at a rate of 9 ml/h at 2–4°C.

Gel filtration yielded two distinct fractions: green and brownish-green. Fractions 6–8 and 19–23 (see Fig. 1) were recombined and used in the preparation of proteoliposomes.

100 mg of soybean phospholipids (asolectin) were suspended in 1 ml of 50 mM sodium phosphate buffer (pH 7.4) containing 4% sodium cholate, and sonicated at 0°C to clarify the suspension. This suspension was mixed with the green or brownish-green fractions of the pigment-protein complexes in a ratio of 1 : 1 (v/v). The mixture was dialyzed for 16 h at 2–4°C in the dark against 200 vol. of 50 mM sodium phosphate buffer (pH 7.4). Dialysis was carried out once more for 24 h against 200 vol. of the same buffer. Proteoliposomes were sedimented by 40 min centrifugation at $16500 \times g$, suspended in 0.5 ml of the same buffer and stored at 2–4°C in the dark.

Absorption spectra of *A. variabilis* membranes and pigment-protein complexes were measured with an SP-14 spectrophotometer. Spectra of light-induced absorbance changes were recorded by means of a differential spectrophotometer described previously [14].

The generation of an electrical potential difference in *A. variabilis* membranes and proteoliposomes was monitored by two methods using membrane vesicles associated with a phospholipid-impregnated Teflon membrane filter in the presence of 20 mM Mg^{2+} [15,16] or penetrating TPB^- or TPP^+, with a phospholipid membrane serving as a penetrating ion-selective electrode [17]. The measurements were carried out in the solution containing 50 mM Tris-HCl buffer (pH 7.6).

PS I and PS II activities of the membranes and pigment-protein complexes were estimated polarographically with a platinum electrode. The chlorophyll content was measured using an extinction coefficient of 60 $mM^{-1} \cdot cm^{-1}$ at 677 nm for pigment-protein complexes of cyanobacteria [18]. A differential extinction coefficient of 64 $mM^{-1} \cdot cm^{-1}$ [19] was used to determine the *P*-700 concentration.

Actinic light with $\lambda > 600$ nm was employed in polarographic studies of photochemical activity and photoelectrical responses. The *P*-700 absorbance changes monitored were induced by light with $\lambda < 600$ nm. The light intensity was adjusted to be saturating.

Results and Discussion

Upon treatment with SDS [18,20], Triton X-100 [21–23] or lauryldimethylamine *N*-oxide [24], cyanobacterial membranes or digitonin-treated subchloroplasts particles produce pigment-protein complexes with a *P*-700 reaction center and light-harvesting antenna Chl *a* at a ratio of 1 : 30–50.

In this study, the pigment-protein complexes isolated from *A. variabilis* membranes by solubilization with lauryldimethylamine *N*-oxide were separated by Sepharose 6B gel filtration into two fractions (Fig. 1). The amount of carotenoids in the first, green, fraction (Fig. 2, curve 2) was smaller than in the second, brownish-green, fraction (Fig. 2, curve 3) and in the isolated membranes (Fig. 2, curve 1). The maxima of the chlorophyll long-wave

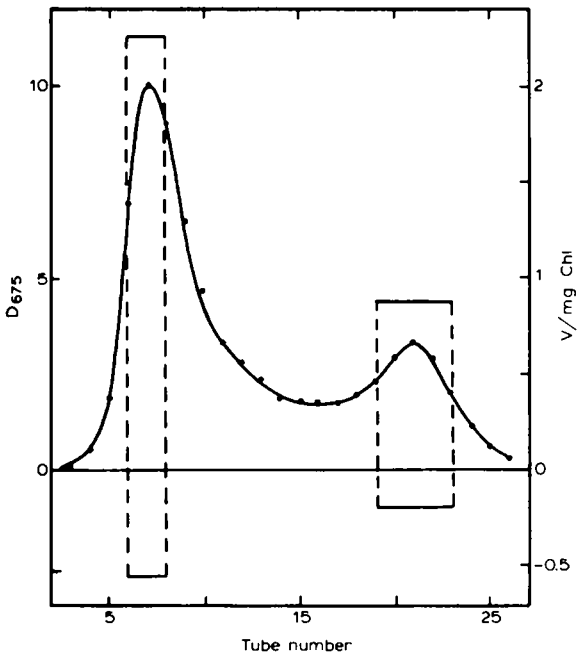

Fig. 1. Chromatography of *A. variabilis* pigment-protein complexes on a Sepharose 6B column. Total fractions 6–8 and 19–23 were used in preparing proteoliposomes. Electrogenic activity of proteoliposomes was measured by the light-induced uptake of TPP^+ (upper columns) and TPB^- (lower columns). TPP^+ and TPB^- uptake is expressed as the electrical potential difference (in volts) across the measuring phospholipid membrane per mg chlorophyll. The incubation mixture contained 0.1 mM phenazine methosulfate , 5 mM Tris-ascorbate and proteoliposomes with a chlorophyll content of 3.2 μg/ml.

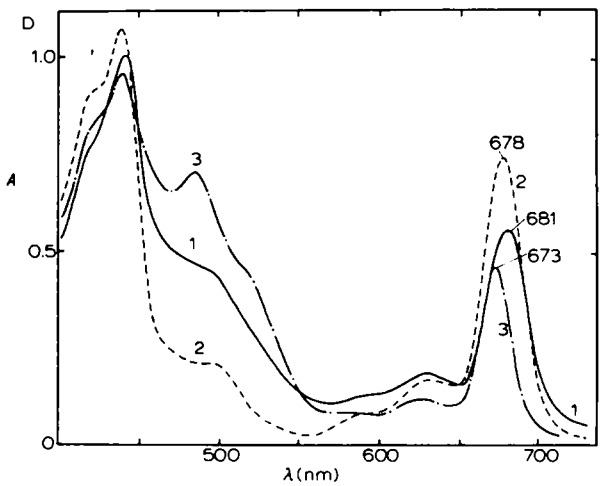

Fig. 2. Absorbance spectra of *A. variabilis* membranes (1), and green (2) and brownish-green (3) pigment-protein complexes from fractions 7 and 21 in Fig. 1.

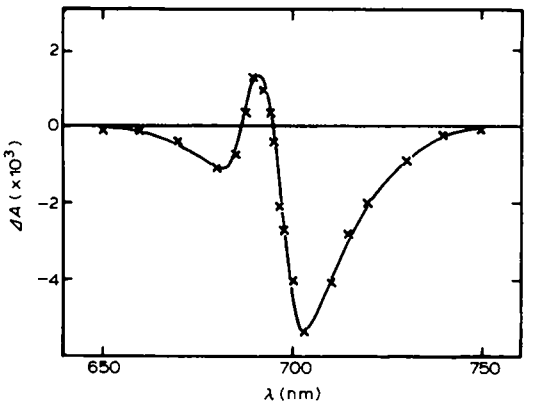

Fig. 3. Light-induced absorbance change spectrum of *A. variabilis* pigment-protein complexes from green fraction 7 in Fig. 1. The absorbance at 678 nm was 0.5 units optical path length 1 cm).

absorption band in the membranes, and the green and brownish-green fractions were located at 681, 678 and 673 nm, respectively.

Both fractions of the pigment-protein complexes possessed *P*-700 activity. A spectrum of *P*-700 light-induced absorbance changes in the case of the green fraction is presented in Fig. 3. Similar spectra were obtained with membranes and the brownish-green fraction (data not shown).

Compared to the isolated membranes, the pigment-protein complexes were enriched in *P*-700 (Table I). The maximum activity of PS I was found in the green fraction and that of PS II in the brownish-green fraction.

Energization of isolated *A. variabilis* membranes by illumination entailed the uptake of penetrating ions, indicative of the generation of an electrical potential difference ($\Delta\psi$) across the membrane vesicles [17]. Uptake of TPB^- by the illuminated membranes was unaffected by ascorbate and stimulated upon the subsequent addition of phenazine methosulfate. Light-dependent uptake of TPP^+ was also observed: this process, in contrast to TPB^- uptake, took place only when ascorbate and phenazine methosulfate were present. The light-induced responses of the penetrating ions were reversed in the dark and inhibited by CCCP, a protonophore uncoupler.

The data on the energy-dependent uptake of both anions and cations suggest the presence of

TABLE I

P-700 CONTENT AND PS I AND PS II ACTIVITY IN *A. VARIABILIS* MEMBRANES AND PIGMENT-PROTEIN COMPLEXES

Additions: 0.1 mM 2,6-dichlorophenolindophenol (DCIP), 2 mM Tris-ascorbate, 1 mM 1,5-diphenylcarbazide (DPC) and 0.2 mM methyl viologen (MV).

Preparation	Chl/*P*-700	Light-induced O_2 uptake (μmol O_2 consumed/mg Chl per h)	
		PS I activity (DCIP+ ascorbate →MV)	PS II activity (DPC →MV)
Membranes	500	49	6.3
Fraction 7 (green)	100	122.5	9.8
Fraction 21 (brownish-green)	150	66	35

two types of membrane vesicles in the preparation obtained from the bacterial cells, by virtue of the orientation of membrane components in the vesicles being opposite. The inside-out (relative to the cytoplasmic membrane) vesicles generating $\Delta\psi$ with a positive charge inside take up TB^-, while those of the other type take up TPP^+, as a result of the opposite direction of the $\Delta\psi$ gradient. Furthermore, it is interesting that the light-induced TPB^- response, unlike the TPP^+ response, was observed in the absence of ascorbate and phenazine methosulfate. This effect may be due to the inside-out vesicles retaining and the right-side-out vesicles losing some water-soluble components (e.g, plastocyanin) participating as electron carriers and being bypassed by phenazine methosulfate.

Fig. 5 shows the photoelectrical responses of the pigment-protein complexes from the green fraction reconstituted into phospholipid vesicles. The pro-

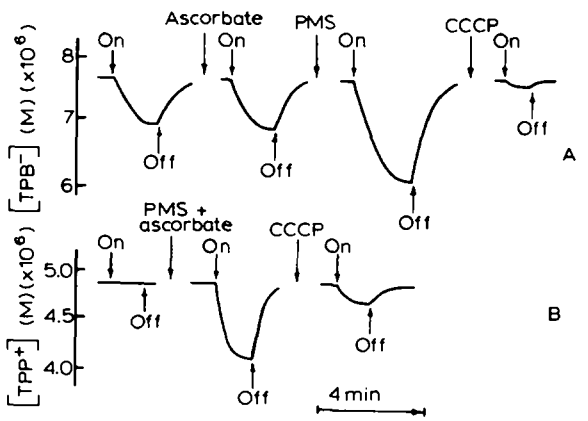

Fig. 4. Light-induced uptake of TPB^- (A) and TPP^+ (B) by *A. variabilis* membrane vesicles. The absorbance at 680 nm was 1.6 units (optical path length 1 cm). Additions: 5 mM Tris-ascorbate, 0.1 mM phenazine methosulfate (PMS) and 5 μM CCCP.

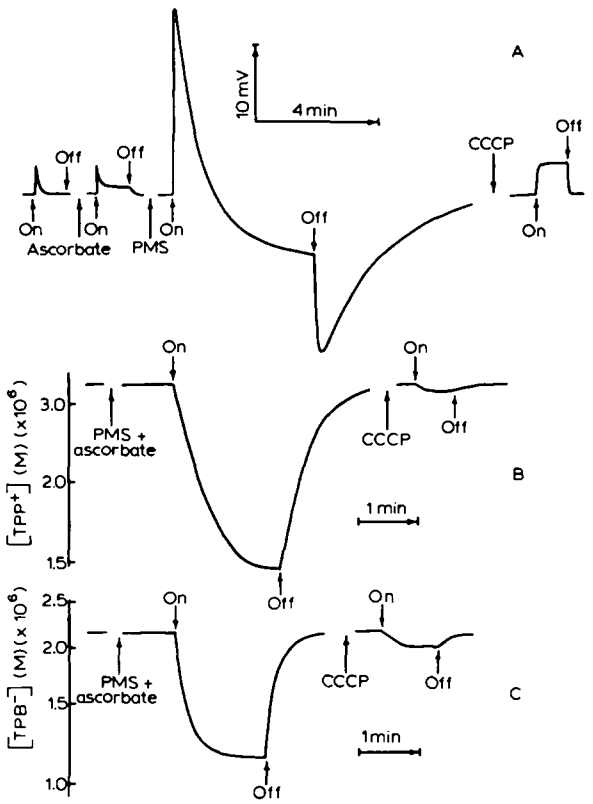

Fig. 5. Photoelectrical responses of proteoliposomes associated with planar phospholipid membrane (A) and light-induced uptake of TPP^+ (B) and TPB^- (C) by proteoliposomes. The proteoliposomes contained *A. variabilis* pigment-protein complexes from the green fraction. Positive charging of the proteoliposome-free compartment in A corresponds to an electrical potential increase. Chlorophyll contents were 4.3, 3.6 and 7.9 μg/ml in A, B and C, respectively. Incubation mixture in A contained 20 mM $MgSO_4$. Additions: 0.1 mM phenazine methosulfate (PMS), 5 mM Tris-ascorbate and 5 μM CCCP.

teoliposomes were added to one of the two compartments separated by a Teflon filter (8 mm diameter, pores size approx. 50 μm) impregnated with a solution of asolectin in decane (100 mg/ml). Thereupon, $MgSO_4$ was added to both compartments. Mg^{2+} causes association of proteoliposomes with the filter, apparently due to neutralization of the negative surface charges of the phospholipids in both the filter and proteoliposomes. Illumination of the proteoliposomes associated with a planar phospholipid membrane gave rise to an electrical potential difference across the planar membrane (Fig. 5A). Addition of ascorbate and phenazine methosulfate produced changes in the magnitude and shape of the photoelectrical response: the electrical potential difference initially reached 25 mV, subsequently decreasing, and reversed its direction. Switching off the light induced a response opposite to that caused by illumination.

The photoelectrical responses observed in the proteoliposome-planar membrane system appear to be due to the generation of two components with opposite orientations: the fast component results from the formation of an electrical potential difference with 'plus' and a slow component with 'minus' inside the proteoliposomes. Both components exhibited CCCP sensitivity (Fig. 5A).

Supportive of the presence of these components, the proteoliposomes incubated with ascorbate and phenazine methosulfate took up both TPP^+ (Fig. 5B) and TPB^- (Fig. 5C) in response to illumination. CCCP was found to be inhibitory to the ion uptake. The inhibition of $\Delta\psi$ generation in the native and artificial (proteoliposome) membranes was observed as soon as CCCP was added, but increased appreciably with time of incubation with the uncoupler and was total at 10–15 min after its addition. Fast and total inhibition of the light-induced responses occurred also with increasing CCCP concentration (data not shown).

The uptake of both penetrating cations and anions by the proteoliposomes, together with the biphasic character of the photoelectrical responses of the proteoliposomes associated with the planar phospholipid membrane, is substantial evidence for the presence of two types of membrane vesicles distinguished by the orientation of the pigment-protein complexes. Possibly, the proteoliposome membrane comprises two types of pigment-protein complexes with opposite sidedness of their membranes.

The electrogenic activity of proteoliposomes containing the complexes from the green and brownish-green fractions is closely related to their elution pattern (Fig. 1). The upper and lower columns show the light-induced uptake of TPP^+ and TPB^-, respectively. Comparison of the data of Fig. 1 and Table I indicates that the electrogenic activity of the proteoliposomes correlated with the PS I reaction center activity.

Thus, we conclude from the results presented above that isolated *A. variabilis* pigment-protein complexes generate a transmembrane electrical potential difference under illumination. The factors governing the orientation of complexes are currently unclear; they provide a subject for further research.

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