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Isolation from *Spirulina* membranes of two photosystem I-type complexes, one of which contains chlorophyll responsible for the 77 K fluorescence band at 760 nm

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Two types of chlorophyll-protein complexes of photosystem I (PSIa, PSIc) have been isolated from the membranes of *Spirulina platensis* using a Triton X-100 treatment and chromatography on DEAE-Toyopearl. The complexes are equally enriched with P700 (Chl: P700=100-110) but show different electrophoretic molecular masses – 140 (PSIa) and 320 kDa (PSIc) – and differ in the content of long-wavelength absorbing Chl. PSIa has a typical PSI fluorescence band at 730 nm (F730) as the main band at 77 K, whereas PSIc is responsible for F760, the intensity of which depends on the redox state of P700. PSIc only shows 77 K light-induced variable fluorescence at 760 typical of *Spirulina* membranes and cells.

Pigment-protein complex of photosystem I; P700; Photosystem I variable fluorescence; Cyanobacteria

1. INTRODUCTION

Photosystem I (PSI) of higher plants shows a low temperature fluorescence band at about 730–735 nm emitted by the chlorophyll (Chl) form absorbing at 705 nm, which is located in Chl *a/b* light harvesting complex I [1–3]. No changes in the quantum yield of F730 correlating with P700 photooxidation, i.e. variable fluorescence of PSI at 77 K, have been observed [4–8]. No correlation with P700 photooxidation was observed also for a fluorescence change at 718–722 nm emitted by the Chl of PSI internal antenna [8]. PSI of cyanobacteria, in contrast to PSI of higher plants, has no light harvesting complex I, rather it has 100 Chl molecules per P700 incorporated in the core complex and internal antenna, and shows a 77 K fluorescence band at 720–725 nm [9]. Along with this band, an unusual 77 K fluorescence band at 750–760 nm was observed in cyanobacterial cells and membranes but not in isolated PSI complex [10,11]. It was shown that F760 is emitted by long-wavelength Chl with an absorption band at 735 nm [12]. The intensity of F760 in the membranes depended on the redox state of P700, namely photooxidation of P700 at 77 K was accompanied by the parallel quenching of F760, probably as a result of energy migration from the Chl_{735}^{760} to oxidized P700 [12]. Here we report on the chromatographic separation of two PSI-type complexes from *Spirulina* membranes which differ in spectral properties and molecular mass. It was found that only the high molecular mass complex contains Chl_{735}^{760} and shows the F760 photobleaching as a result of P700 photooxidation.

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Abbreviations: Chl, chlorophyll; Chl_{735}^{760}, Chl *a* with absorption maximum at 735 nm and fluorescence maximum at 760 nm; F730, F760 fluorescence bands with maximum at 730 or 760 nm; PSI, photosystem I; P700, primary electron donor of PSI; PMS, phenazine methosulfate.

2. MATERIALS AND METHODS

Cells and membranes of *Spirulina platensis* were grown and prepared as described in [12]. Membranes were washed twice with 50 mM Tris-HCl buffer (pH 7.8) containing 5 mM $MgCl_2$, 10 mM NaCl, and once with buffer containing 0.1% Triton X-100. The washed membranes were solubilized with 2% Triton X-100 (detergent: Chl=20 mg/mg) using the same buffer. Non-solubilized membranes were removed by centrifugation at 100,000 × *g* for 1 h at 4°C. The supernatant (6–8 mg of Chl) was applied to DEAE-Toyopearl column (1.5 × 10 cm) equilibrated with 10 mM Tris-HCl buffer (pH 7.8) containing 0.05% Triton X-100 (buffer A). The column was washed with 4–5 vols. of buffer A and was then subjected to a linear NaCl gradient from 0 to 300 mM in buffer A. For re-chromatography the combined fractions at about 120 and 240 mM NaCl were initially diluted 2.5-fold in buffer A and then loaded onto the same DEAE-Toyopearl column.

Protein composition was assayed by SDS-PAGE on a 7.5–15% slab gel at 4°C [13] and stained with Coomassie R-250. For 'native' electrophoresis the isolated complexes in buffer A were loaded directly on the gel without heating.

Absorption spectra, fluorescence emission spectra (corrected for spectral sensitivity) and light-induced absorption and fluorescence changes at 77 K were all measured using an Hitachi-557 spectrophotometer and MPF-4 spectrofluorimeter as described in [12]. The Chl and P700 concentrations were detected as in [12].

3. RESULTS

About 70% of the Chl is extracted upon mild solubil-

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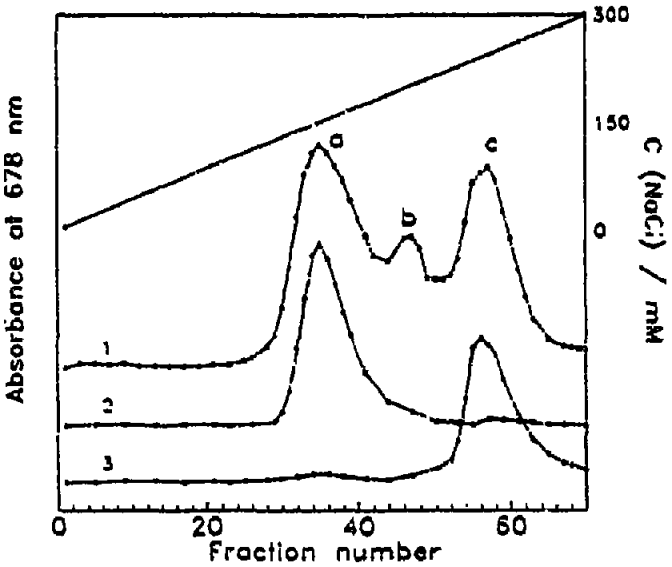

Fig. 1. Elution profiles of PSI complex preparations from the anion-exchange column, DEAE-Toyopearl, by a NaCl gradient in buffer A; flow rate 1.5 ml/min. Curve 1, initial mixture; curves 2 and 3, re-chromatography of peaks a and c.

ization of *Spirulina* membranes with Triton X-100, and supernatant mainly contains PSI proteins. Free Chl is essentially absent and total Chl is adsorbed on the DEAE-Toyopearl. Three Chl-containing fractions are eluted at about 120, 170 and 240 mM NaCl (Fig. 1,

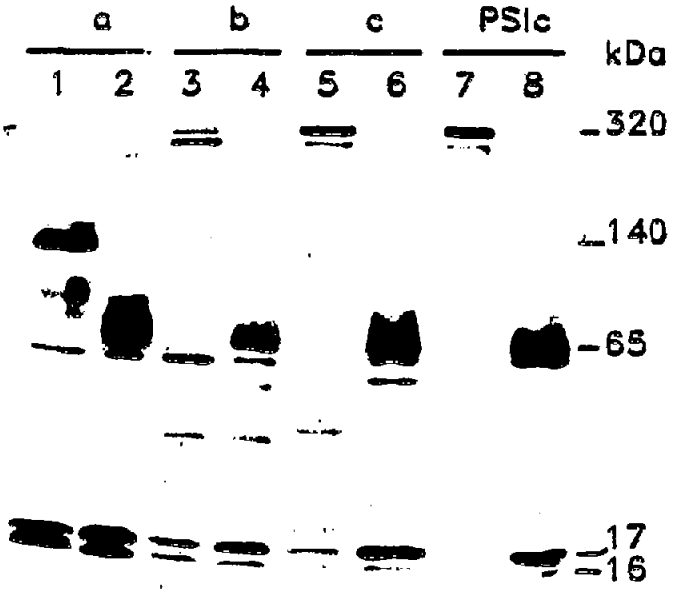

Fig. 2. Electrophoresis of the PSI complexes on 7.5–15% SDS-polyacrylamide gels. (Tracks a, b and c) The fractions a, b and c from the DEAE-Toyopearl column (Fig. 1, curve 1). (Track PSIc) Fraction c after repeated chromatography (Fig. 1, curve 3). Tracks 1, 3, 5, 7 were obtained under conditions of 'native' electrophoresis (see section 2); tracks 2, 4, 6 and 8 represent polypeptide profiles of the same preparations.

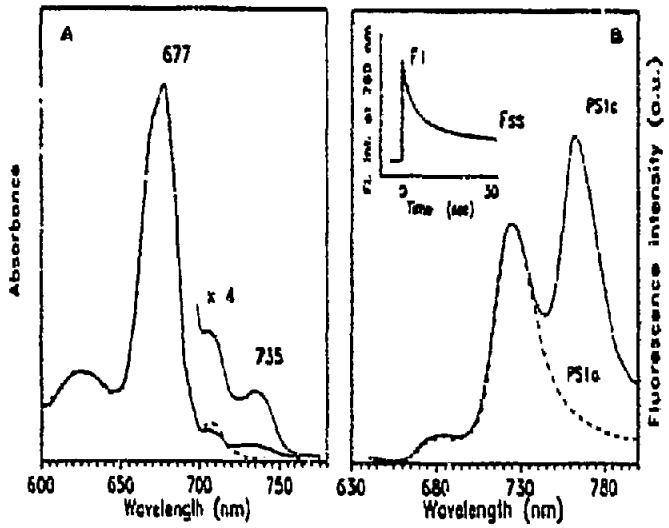

Fig. 3. 77 K absorption (A) and fluorescence emission (B) spectra of PSIa and PSIc complexes frozen in the dark with 20 mM sodium ascorbate (λ_{ex} = 440 nm, 10 mW/m^2). Inset: time-course of F760 photobleaching of dark frozen PSIc at the same excitation.

curve 1). The quantity of Chl in fractions a and c was approximately equal and varied little in different experiments. Complexes in fractions a and c (PSIa and PSIc) are stable to re-chromatography (curve 2 and 3): the ratio Chl:P700 is 100–110 for both complexes.

According to electrophoretic data (Fig. 2), PSIa and PSIc complexes have similar polypeptide compositions: 65, 17, 16.3, and 16 kDa, but PSIc is poor in 16 and 16.3 kDa proteins (tracks 2, 4, 6 and 8). Under conditions for 'native' electrophoresis (tracks 1, 3, 5 and 7) the apparent molecular mass of PSIa (monomer) and PSIc (oligomer) complexes was about 140 and 320 kDa. The contamination of proteins with molecular masses 51, 47 and 32 kDa was absent after re-chromotography (tracks 7 and 8).

In contrast to PSI complexes from *Synechococcus* spp. with different molecular masses [14,15], we have found a significant spectral difference between monomeric and oligomeric PSI complexes: Chl_{735}^{200} specific for *Spirulina* is completely absent in PSIa. PSIc has a more intense absorption band at about 735 nm and a fluorescence band at about 762 nm (Fig. 3) compared with the membranes. The fluorescence band at 726–728 nm (F730) typical of *Spirulina* membranes was conserved in the spectra of both complexes. Weak fluorescence bands at 685 and 693 nm were screened by emission of free Chl at 680 nm whose relative intensity is reduced by 2.5–3 during excitation at 480–490 nm. Characteristically for membranes the narrow (14–15 nm) 77 K absorption band at 710 nm was reduced in both complexes.

Illumination by monochromatic light of dark-frozen PSIc complexes in the presence of 3 mM sodium ascorbate causes the negative variable fluorescence-F760

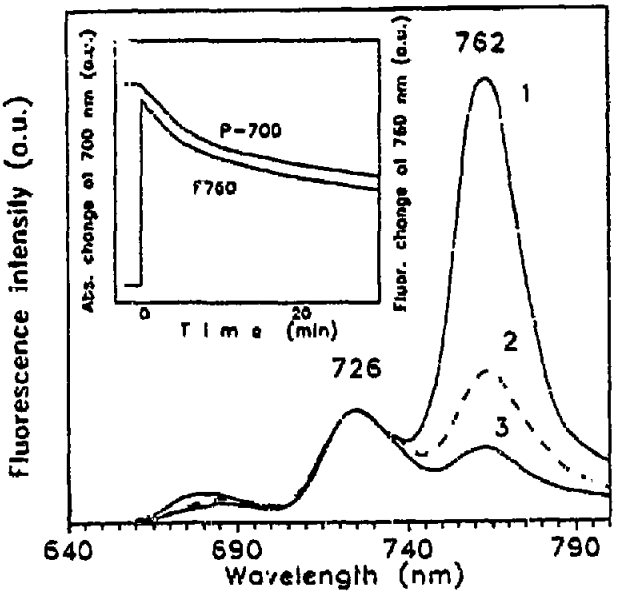

Fig. 4. 77 K fluorescence emission spectra of PSIc complexes (λ_{ex} = 440 nm). Curve 1, complex frozen upon illumination with white light (10 W/m^2), in 100 mM glycine–NaOH buffer (pH 9.5), 20 mM dithionite and 10 μM PMS; curves 2 and 3, complex frozen in the dark in the same buffer in the presence of 10 mM sodium ascorbate and 10 μM of PMS (2) or 3 mM ferricyanide (3). Inset: time-course of F760 quenching and P700 oxidation measured under identical conditions; intensity of the exciting (measuring) beam at 702 nm was 3 mW/m^2.

quenching (Fig. 3, inset): the fluorescence intensity at 760 nm is reduced 3.5–4 times from the initial (F_i) to the steady-state level (F_{ss}). The fluorescence spectra presented in Fig. 3B correspond to the F_{ss} level.

As for membranes, the relative intensity of F760 in PSIc complexes depends on the redox state of the reaction centers: it is maximal and stable (probably F_i level) if P700 and PSI acceptor side are pre-reduced by illumination with strong light in the presence of dithionite during cooling (Fig. 4, curve 1) and it is minimal in the oxidative condition, induced by ferricyanide (Fig. 4, curve 3). However, in contrast to membranes, the F760 band in the PSIc preparation is not quenched completely by ferricyanide. As in the membranes [12], the kinetics of F760 photobleaching coincides completely with the kinetics of P700 photooxidation (Fig. 4, inset).

4. DISCUSSION

The results show that an unusual chlorophyll Chl_{735}^{760} typical of the cells (and membranes) of some cyanobacteria was stable against detergent treatment and conserved in isolated PSI complexes. It was present only in the oligomeric PSIc complex and it is very likely that the formation of Chl_{735}^{760} is induced specifically for cyanobacteria by oligomerization of PSIa. This might be an indication that oligomeric (PSIc) complexes pre-exist in the *Spirulina* membranes and that this oligomerization is therefore not a result of detergent treatment. The absence of the Chl in the monomeric PSIa complex can not be a result of its degradation by detergent, as the oligomeric PSIc complexes are enriched with Chl_{735}^{760} as compared with the membranes.

The mechanism of F760 photobleaching is still unclear. Duval et al. [10,11] proposed that the quenching of F750 may result from recombination between reduced acceptors and P700$^+$. Earlier we suggested that light-induced quenching of F760 is due to the energy migration from Chl_{735}^{760} on the cation radical of P700 [12]. The observation that F760 in the PSIc complex in the presence of ferricyanide is strongly reduced (Fig. 4, curve 3) but not completely eliminated, as in membranes [12], may give additional argument to the energy migration mechanism. In fact, this difference between properties of F760 in the isolated complex and membrane could be explained by the breaking of contact between P700 and Chl_{735}^{760} after detergent treatment. The role of Chl_{735}^{760} in PSI functioning is not yet clear, but the strong relationship between that and P700 is obvious. Chl_{735}^{760} may serve as an energy donor to P700 and, due to the efficient energy transfer from Chl_{735}^{760} to P700$^+$, promote the process of energy dissipation in PSI after photooxidation of P700. In any case this PSIc complex offers new approaches to study the energy pathways in PSI.

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