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Trimeric forms of the photosystem I reaction center complex pre-exist in the membranes of the cyanobacterium *Spirulina platensis*

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Oligomeric and monomeric forms of chlorophyll–protein complexes of photosystem I (PSI) have been isolated from the mesophilic cyanobacterium *Spirulina* [(1992) FEBS Lett. 309, 340–342]. Electron microscopic analysis of the complexes showed that the oligomeric form is a trimer of the shape and dimensions similar to those of the trimer from thermophilic cyanobacteria. The chlorophyl ratio in the isolated trimer and monomer was found to be 7:3. The trimeric form of PSI complex in contrast to the monomeric one contains the chlorophyll emitting at 760 nm (77K), which is also found in *Spirulina* membranes and therefore could be used as an intrinsic probe for the trimeric complex. The 77K circular dichroism spectrum of the trimeric form is much more similar to that of *Spirulina* membranes than the spectrum of the monomer. Thus, the trimeric PSI complexes exist and dominate in the *Spirulina* membranes.

Pigment–protein complex of photosystem I; Trimer; Electron microscopy; Cyanobacteria; Chlorophyll; Circular dichroism

1. INTRODUCTION

Photosystem I (PSI) complexes isolated from membranes of higher plants, alga and cyanobacteria were described as monomers consisting of two Chl-binding subunits with a molecular mass of 82 and 83 kDa, 9–10 low-molecular weight polypeptides (2.5–18 kDa), and about 100 molecules of Chl per P700 [1]. In addition to monomeric PSI complexes, a number of oligomeric PSI complexes were isolated from cyanobacteria [2,3]. The oligomeric PSI complex of thermophilic strains of *Synechococcus sp.* and *Phormidium laminosum* is mainly a trimer which according to electron microscopy is a flat, disk-shaped structure 19 nm in diameter and 8–10 nm thick [4–7].

Since there is no evidence that the PSI trimer really exists in cyanobacterial membranes, the presence of the trimeric PSI complexes in the preparations of PSI complexes was mainly explained in two ways. Some authors suggested that the PSI complex existed in membranes as a monomer, and oligomers are formed during the isolation of the complex as a result of the high affinity of monomers for aggregation [6,7], while the other believed that because of the high resistance of oligomers to detergents, trimeric PSI might preexist in membranes [4,5]; the yield of oligomeric PSI complexes (probably trimers) increased in the course of isolation as a result of chemical cross-linking of proteins before solubilization of cyanobacterial membranes [8]. Preexistence of an oligomeric PSI in *Spirulina* membranes was also suggested by the fact that the oligomeric PSI fraction contained a special Chl (Chl_{735}^{760}) whose fluorescence yield depended on the redox state of P700 [9,10].

The evidence for the existence of trimeric PSI complexes in cyanobacterial membrane is extremely important in connection with X-ray analysis of the molecular structure of the PSI trimer isolated from the cyanobacterium *Synechococcus sp.* [11]. This is also necessary for studying the role of the trimer in energy conversion in PSI reaction center of cyanobacteria by monitoring the fluorescence yield at 760 nm depending on the redox state of P700 [9,10]. This paper gives the evidence that trimeric forms of PSI complexes preexist and predominate in membranes of the mesophilic cyanobacterium *Spirulina*; the structure of the trimer is identical to that of the trimeric forms of PSI complexes isolated from thermophilic cyanobacteria [4–7].

2. MATERIALS AND METHODS

Cells of the cyanobacterium *Spirulina platensis* were grown and the membranes were prepared as described in [9,10]. After Triton X-100 treatment (detergent:Chl = 20 mg/mg), nonsolubilized membranes were removed by centrifugation at 50,000 × *g* for 40 min at 4°C. Isolation of monomeric and oligomeric PSI complexes was carried out using DEAE-Toyopearl column according to [11]. SDS-PAGE was performed on 4.5% slab gel at 4°C [11]. The membranes solubilized with Triton X-100 and isolated PSI complexes were mixed with SDS (detergent:Chl = 3 mg/mg) before electrophoresis.

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Abbreviations: Chl, chlorophyll; Chl_{735}^{760}, Chl *a* with absorption maximum at 735 and fluorescence maximum at 760 nm; CD, circular dichroism; PS, photosystem; P700, primary electron donor of PSI; SDS-PAGE, SDS-polyacrylamide gel electrophoresis.

Absorption, fluorescence emission spectra and CD spectra at 77K were all measured using a Hitachi 557 spectrophotometer, MPF-4 spectrofluorimeter and Jasco-40AS dichrograph, respectively (2-mm pathlength plastic cuvette). CD spectra are presented as $\Delta A = A_l - A_r$, the rotational strength was characterized as $\Delta A/A$ (A, absorbance). The membranes and isolated PSI complexes suspended in a 20 mM Tris-HCl buffer (pH 7.8) were frozen to the glassy state in 60% glycerol. The light scattering of the membranes was insignificant and CD spectra did not depend on the distance (3–20 cm) from the photomultiplier. For electron microscopy purified PSI complexes diluted with 50 mM Tris-HCl buffer (pH 7.8) plus 0.05% Triton X-100, were applied to thin colloidon films and negatively stained with 1% uranyl acetate. Grids were examined in a Philips EM400 electron microscope at 80 kV. Electron micrographs were recorded at calibrated magnifications (× 50,000).

3. RESULTS

Previously, it was found that the relative distribution of Chls in oligomeric and monomeric PSI complexes (calculated from an elution profile of the DEAE-Toyopearl column) was 2:3 after solubilization of *Spirulina* membranes with Triton X-100 [11]. The ratio changed to 7:3 if the unsolubilized membrane fraction was removed by centrifugation at 50,000 × g for 40 min instead of 100,000 × g for 60 min. A similar ratio was obtained from densitometric traces of nonstained gel after electrophoretic separation of Chl protein complexes from the membranes solubilized with Triton X-100 (Fig. 1) and SDS (data not shown) at detergent:Chl ratios 20, 30 and 50. The band between PSI trimer and monomer could be due to PSI dimer (Fig. 1) which then dissociated.

The size of oligomeric complexes from *Spirulina* membranes was determined from an electron micrograph of PSI complexes separated on DEAE-Toyopearl

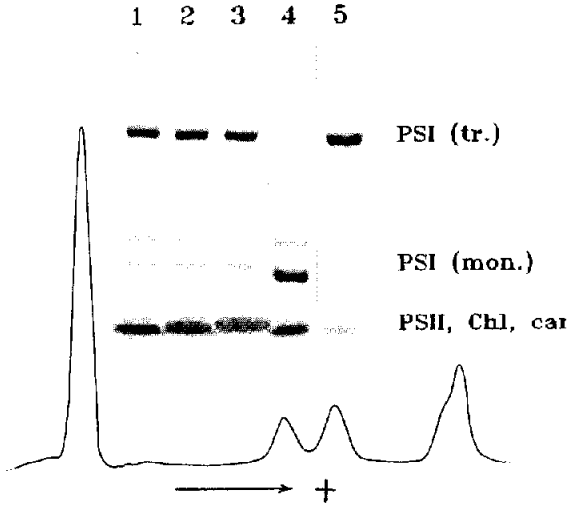

Fig. 1. Electrophoretic separation of the PSI complexes obtained by solubilization of *Spirulina* membranes with Triton X-100 on 4.5% polyacrylamide (not stained). Lanes 1–3, Triton extracts with the ratio of detergent:Chl of 20, 30 and 50, respectively; lanes 4 and 5, the fractions of oligomeric and monomeric PSI complexes from DEAE-Toyopearl column. The densitometric scan is of track 2 at 670 nm.

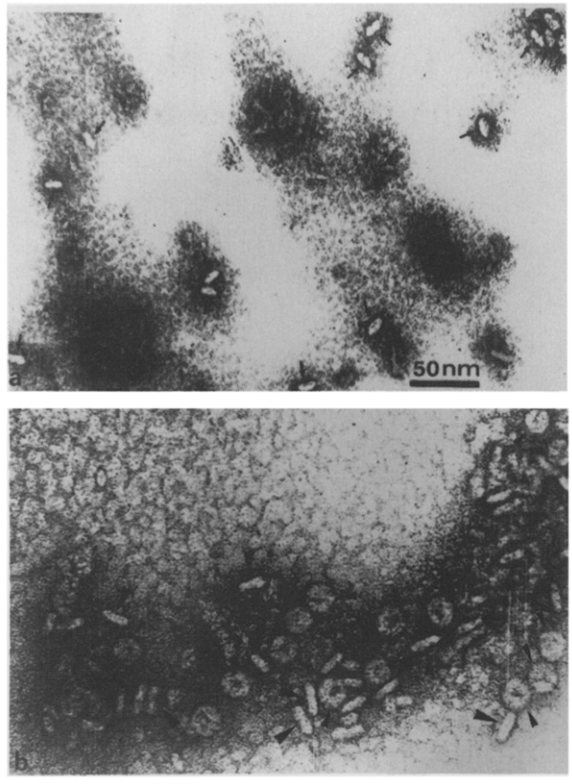

Fig. 2. Electron micrographs of negatively stained PSI complex preparations. (Upper panel) Monomeric fraction. (Lower panel) Oligomeric fraction. The top- and side-view projections of the particles are indicated by small and large arrowheads, respectively.

column as was done in [11]. The general view of the negatively stained monomeric and oligomeric fractions of PSI complexes is shown in Fig. 2. The monomeric fraction (Fig. 2, upper panel) has longitudinal particles about 15 nm long and 7.5–8 nm wide with two domains. These particles are similar to monomeric PSI complexes consisting of two Chl-binding polypeptides of about 80 kDa [12].

In micrographs of the oligomeric fraction (Fig. 2, lower panel), two distinct types of particle projection could be readily observed. The particle projections of the first type, indicated by small arrowheads, are of circular shape about 19–20 nm in diameter. The projections of the second type (Fig. 2, lower panel, large arrowheads) measure (19–20) × (6– 7) nm; their length is equal to the diameter of circular particles. The particles observed are very similar to the trimeric molecular form described for PSI complexes isolated from thermophilic strains of *Synechococcus sp.* [4,5] and *Ph. laminosum* [6,7]. They are disk-shaped and most frequently visible in the projections: the images of the first and second types (Fig. 2, lower panel) correspond to the top- and side-view particle projections, respectively.

An approach to solving the problem of the existence of monomeric and trimeric PSI complexes in cyanobacterial membranes is to compare the spectral properties of pigments in these samples. Similar to *Synechocys-*

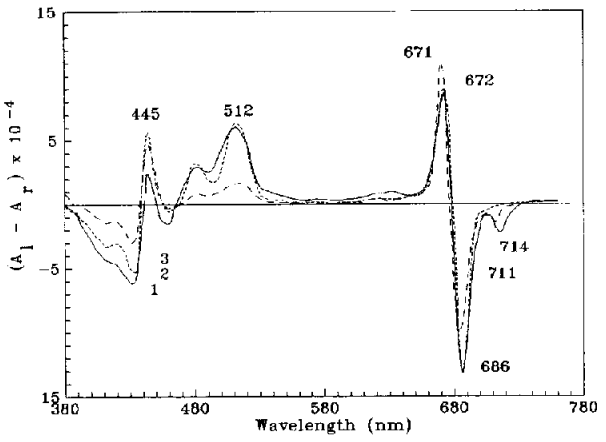

Fig. 3. 77K CD spectra of the *Spirulina* membranes (1) and isolated trimeric (2) and monomeric (3) PSI complexes. For all samples $A = 1$ at 678 nm.

tis membranes [13], *Spirulina* membranes (Fig. 1) contain at least five times more PSI than PSII, and spectral properties of the membranes should be determined mainly by PSI. It was shown earlier that oligomeric (trimeric) and monomeric PSI complexes significantly differed in the region of Q_y transition of Chl; Chl_{735}^{760} with a half-bandwidth of 32–34 nm is only present in the trimer, while nonfluorescent Chl_{711} with a half-bandwith of 14 nm is found both in the trimer and in the monomer [9]. However, the comparison of CD (Fig. 3) and absorption spectra at low temperature shows that Chl_{711} has a different organization in the monomer and trimer. The rotational strength of 711 nm band in the monomer is significantly higher than in the trimer: anisotropy factor ($\Delta A/A$) at 711 nm is 6 times more in the monomer than in the trimer (Table I). The bands 711 and 735 nm in the CD spectrum of the trimer are of such low intensity that they could not be accurately registered because of alterations of the zero line in the course of low temperature measurements.

According to the second derivative of 77K spectra, the PSI monomer and trimer differ in the ratio of the main absorption bands of antenna Chls at 662, 670, 676, 680, 686 and 695 nm with a half-bandwidth of 7 8 nm (data not shown). The 77K CD spectrum of *Spirulina* membranes is very similar to the CD spectra of the trimeric PSI complex, but significantly differs from those of the monomeric PSI complex. The values of the $\Delta A/A$ at 672 and 686 nm (maximum and minimum for bulk antenna Chl in the CD spectra), and the $\Delta A_{672}/\Delta A_{686}$ ratio is very close for the membranes and the trimer (Table I). The $\Delta A_{512}/\Delta A_{686}$ ratio (512 nm is the maximum for carotenoids) is equal for the membranes and the trimer and sharply differs from that of the PSI monomer. The different values of $\Delta A/A$ at 512 nm for membranes and trimeric PSI complexes seem to have resulted from the presence of an additional amount of optically inactive carotenoids in the membranes. It is noteworthy that the values of the $\Delta A/A$ at 711 nm are identical for membranes and monomeric PSI and 6–8 times higher than that of trimeric PSI complexes (Table I). However, Chl_{711} is partially destroyed after detergent treatment, since its relative intensity in absorption and CD spectra always decreases in isolated complexes.

4. DISCUSSION

The results obtained indicate that most PSI complexes isolated from mesophilic *Spirulina* are disk-shaped trimers similar to those from the thermophilic cyanobacteria *Synechococcus sp.* and *Ph. laminosum* [4–7,9]. PSI oligomers found electrophoretically in a number of cyanobacteria [2,3,13,14] are likely to be trimers as well. The predominance of PSI in cyanobacteria and spectral differences between monomeric and trimeric forms of PSI complexes allows us to conclude that trimeric PSI complexes may preexist in *Spirulina* membranes. Chl is an internal marker, which reflects the spatial organization of PSI, since the complicated structure of the CD, absorption and fluorescence spectra is due to the exciton coupling of specially oriented dipole transitions [15]. Therefore, the similarity of the CD spectra of the membranes and isolated trimeric PSI complexes is very difficult to explain as a coincidence. The rotational strength of Q_y transition of Chl of PSII core complex [16] is 3–4 times less than in PSI complex and the CD spectrum of membranes is mainly due to PSI complexes.

The similarity of the CD spectra of antenna Chls in the membranes and PSI trimer and the presence of unusual Chl_{735}^{760} only in PSI trimer are in accordance with chromatographic and electrophoretic data on the predominance (more than 70%) of the trimeric PSI in *Spirulina* membrane and confirm a number of arguments

Table I

The values of the anisotropy factor ($\Delta A/A$) and the ratio of main optically active chlorophylls and carotenoids, calculated from 77K CD and absorption spectra of *Spirulina* membranes and isolated monomeric and trimeric PSI complexes

Sample	Anisotropy factor ($\times 10^3$)				$\Delta A_{711}/\Delta A_{686}$	$\Delta A_{672}/\Delta A_{686}$	$\Delta A_{512}/\Delta A_{686}$
	711 nm	686 nm	672 nm	512 nm			
Membrane	−1.7	−2.3	0.96	1.4	0.17	−0.65	−0.46
Trimer	−0.3	−2.4	0.95	2.3	0.02	−0.66	−0.48
Monomer	−1.8	−1.8	0.85	0.4	0.14	−1.08	−0.10

about the existence of trimers in cyanobacterial cells [14]. It is noteworthy that the high rotational strength of the carotenoid band at 512 nm is not a distinctive feature of trimeric organization of PSI, since the analogous CD signal was observed for monomeric PSI complexes isolated from higher plants [17]. Our data also indicate that a certain amount of monomeric PSI is probably present in *Spirulina* membranes. Indeed, it is difficult to suggest that the anisotropy factor at 712 nm may increase 6 times after dissociation of trimeric PSI complexes and coincide with that of this band in membranes.

Thus, spectral analysis of pigments of *Spirulina* membranes and isolated PSI complexes, as well as the chromatographic and electrophoretic data indicate that trimeric PSI complexes preexist and predominate in *Spirulina* membranes. Since the PSI trimer preexists in cyanobacterial membranes, X-ray data [11] reflect the real molecular organization of PSI complex in vivo.

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