Purification and composition of photosystem I reaction center of *Prochloron* sp., an oxygen-evolving prokaryote containing chlorophyll *b*

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The photosystem I reaction center complex of the photosynthetic prokaryote *Prochloron*, present as a symbiont in ascidians from the Red Sea at Eilat (Israel), was isolated and characterized. The complex consists of 4 polypeptide subunits, and therefore is similar to that of cyanophytes and green algae. Their apparent molecular masses (in kDa) are about 70 (subunit I), 16 (subunit II), 10 (subunit III), and 8 (subunit IV). The purified reaction center contains about 40 chlorophyll molecules per P-700 as compared to about 800 in the intact thylakoids. Subunit I has the same apparent electrophoretic mobility and appears as a double polypeptide band as reported for this subunit in higher plants and algae. Immunological cross-reactivity was detected among subunits I and II of *Prochloron* and photosystem I reaction centers of higher plants and algae.

Photosystem I *Reaction center subunit* Prochloron *P-700*

1. INTRODUCTION

Prochloron is a unicellular prokaryote exhibiting oxygenic photosynthesis which occurs exclusively in association with certain didemnid ascidians [1]. It resembles eukaryotic chloroplasts in that it possesses chlorophyll *b* [2,3], partially stacked thylakoids [4], and lacks phycobilisomes and phycobiliproteins [3]. The *Prochloron* chlorophyll *a/b*-protein complex associated with PS II contains one major polypeptide of 34 kDa [5,6] which is phosphorylated in vitro by a membrane-bound, light-insensitive kinase [5]. In contrast, *Prochloron* resembles cyanobacteria in its prokaryotic cellular organization [7], 16 S ribosomal RNA sequence homologies [8,9], cell wall structure, and carotenoid and lipid composition [10]. The possibility of its relationship to the ancestor of the eukaryotic chloroplast has been considered by many authors [1–11].

In a previous work, the polypeptide composition and organization of chlorophyll-protein complexes of *Prochloron* were compared with those of eukaryotic chloroplasts [5]. Since differences were found in the PS II complex, it was of interest to obtain information on PS I as well. Here, we describe the purification of PS I RC from *Prochloron*, its subunit composition, ratio of chlorophyll/P-700, and immunological cross-reactivity with subunits I and II of PS I RC of higher plants.

2. MATERIALS AND METHODS

2.1. *Analytical methods*

Chlorophyll was measured according to Arnon [12]. P-700 was determined as described [13]. Gel

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Abbreviations: PS, photosystem; RC, reaction center; SDS-PAGE, SDS-polyacrylamide gel electrophoresis

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electrophoresis in slab gels containing an exponential gradient of 10–15% acrylamide was performed according to Douglas and Butow [14]. Transfer from slab gels to nitrocellulose paper and immunodecoration by antibodies, as well as ^{125}I-protein A, were performed as in [15]. The specific antibodies against denatured membrane proteins were obtained and tested as described [15,16].

2.2. *Isolation of cells and preparation of thylakoids*

Prochloron cells were isolated from the didemnid *Diplosoma virens* collected in the Gulf of Aqaba near the Marine Biological Laboratory in Eilat, Israel, at a depth of about 30 m [5]. Cells were released from the ascidians by gentle pressure and flushing with seawater, containing 10 mM Tricine buffer. The cells were collected by centrifugation at 3000 × *g* for 2 min at 4°C.

Preparation of thylakoids was carried out as in [5]. The thylakoid fraction was first washed by centrifugation in 10 mM Tricine-NaOH buffer, pH 8.0, then in the same buffer containing 150 mM NaCl, and finally once more in Tricine-NaOH buffer as above [14]. The washed thylakoids were used for isolation of the PS I RC as described below.

3. RESULTS AND DISCUSSION

3.1. *Isolation of PS I RC*

Octyl-β-D-glucopyranoside and sodium cholate were added to the washed thylakoids at a final concentration of 1 and 0.5%, respectively. After 20 min at 0°C, the suspension was centrifuged at 200000 × *g* for 1 h. The pellet was homogenized in a solution containing 25 mM Tricine-NaOH, pH 8.0, and 2% Triton X-100, to give 0.5 mg chlorophyll/ml [17]. After centrifugation at 20000 × *g* for 10 min, the supernatant was applied on a DEAE-cellulose column (0.6 × 10 cm); the column was washed with 20 mM Tris-HCl, pH 8.0, and 0.2% Triton X-100, and eluted with the same solution containing 150 mM NaCl. A dark-green fraction was collected and applied on a linear sucrose gradient (5–30%) in a solution containing 20 mM Tris-HCl, pH 8.0, and 0.5% Triton X-100. Following centrifugation for 15 h at 150000 × *g* in an SW-41 rotor, 4 green bands appeared. After analysing their absorption spectrum and P-700 content, it was found that the 2 lower bands, designated as Pa (the heavier one) and Pb (the lighter one), contained P-700 activity (fig.1, table 1); hence, these bands were referred to as PS I RC. Table 1 shows the purification of the 2 PS I RC fractions with respect to chlorophyll and P-700 content. While the ratio chlorophyll/P-700 was about 780 (mol/mol) in *Prochloron* thylakoids, it was reduced to 123 and 43 in the Pa and Pb fractions, respectively. The amount of chlorophyll molecules associated with Pa and Pb PS I RC was the only difference found between these two complexes.

3.2. *Polypeptide composition*

Fig.2 shows the polypeptide composition of the 2 purified complexes Pa and Pb, both consisting of the same 4 subunits, with apparent molecular masses of 70, 16, 10 and 8 kDa, further referred to as *Prochloron* PS I RC subunits I–IV, respectively. The polypeptide pattern of purified *Prochloron* PS I RC was also compared to those of *Chlamydomonas, Dunaliella* and spinach PS I RCs. Subunit I had a similar electrophoretic mobility in all preparations and appeared as a dif-

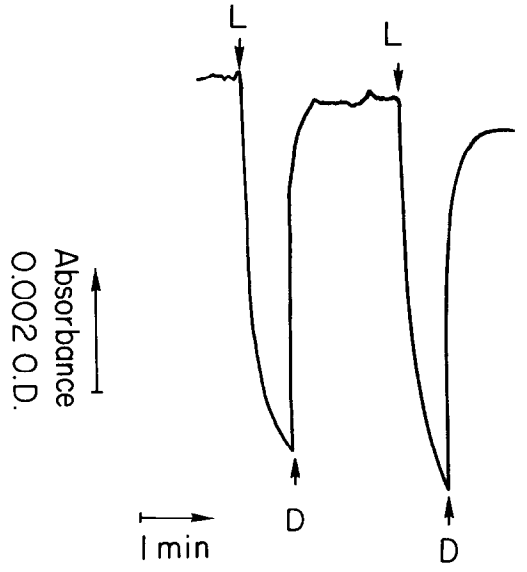

Fig.1. Photo-oxidation of P-700 in *Prochloron* PS I RC. The reaction mixture contained, in a final volume of 200 μl, 2 nmol sodium ascorbate, 0.6 μg *N*-methylphenazonium methosulfate, and PS I RC (Pb) equivalent to 10 μg chlorophyll. Light-induced absorbance changes were recorded at 430 nm, as in [19].

Table 1

Purification of PS I reaction center from *Prochloron*

Purification step	Total chlorophyll (mg)	Total P-700 (nmol)	Chlorophyll/ P-700 (mol/mol)	P-700 recovery (%)
(1) Thylakoids	2.62	3.50	786	100
(2) After octylglucoside and cholate treatment	1.06	1.76	627	50
(3a) PS I RC (sucrose gradient, lower fraction) Pa	0.027	0.23	123	6.6
(3b) PS I RC (sucrose gradient, upper fraction) Pb	0.014	0.34	43	9.7

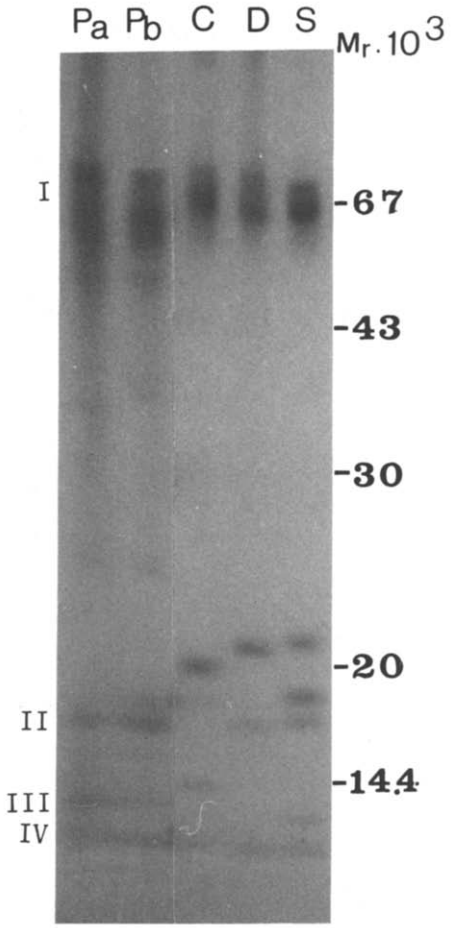

Fig.2. SDS-PAGE of purified PS I RC from spinach (S), *Dunaliella* (D), *Chlamydomonas* (C) and *Prochloron* (P). Pa, the heavier PS I RC fraction in the sucrose gradient; Pb, the lighter PS I RC band in the sucrose gradient. Spinach PS I RC was prepared as in [17]. *Dunaliella* and *Chlamydomonas* PS I RCs were purified according to [19]. The preparations were dissociated for about 2 h at room temperature in the presence of 2% SDS and 2% 2-β-mercaptoethanol, and were electrophoresed on an exponential 10–15% acrylamide gel. The various subunits of the *Prochloron* RC are indicated to the left.

fuse double band of about 70 kDa (fig.2). Furthermore, subunit I cross-reacted with antibodies raised against subunit I of Swiss chard (fig.3), thus

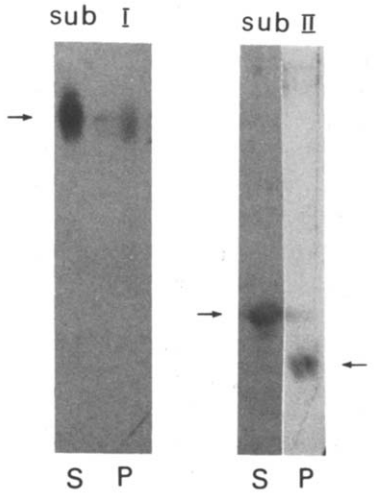

Fig.3. Immunological cross-reactivity among subunits I and II of spinach (S) and *Prochloron* (P) PS I RCs. RCs of spinach and *Prochloron* were electrophoresed on a 10–15% acrylamide gel. The protein bands were electrotransferred to nitrocellulose paper and decorated by a specific antibody against subunit I or subunit II of Swiss chard PS I RC and ^{125}I-protein A as described [15,16].

being homologous to the P-700 polypeptide of eukaryotic chloroplasts.

The second subunit of *Prochloron* PS I RC has a higher electrophoretic mobility than subunit II of all the other preparations tested in this work (16 kDa vs 20–22 kDa) but cross-reacts with anti-Swiss chard subunit II antibodies (figs 2 and 3). Since these antibodies also react specifically with subunit II of spinach (fig.3), *Chlamydomonas* and the cyanophyte *Mastigocladus* [18], it is reasonable to conclude that the *Prochloron* PS I RC subunit II is also homologous to subunit II of both eukaryotes' and cyanophytes' PS I RC.

The third subunit of *Prochloron* (10 kDa, fig.2) has an electrophoretic mobility similar to that of subunit IV of higher plants which have a PS I RC composed of 7 subunits [17], and differs from subunit III of *Chlamydomonas* as well, which possesses a complex containing 4 polypeptide subunits like *Prochloron*. A similar situation is found for the last subunit (IV) of *Prochloron* PS I RC, which shows an electrophoretic mobility comparable to that of subunit VII of spinach and IV of both *Chlamydomonas* and *Dunaliella*. Based on these data, one cannot yet ascertain possible homologies between subunits III and IV of PS I RC of *Prochloron* and those of other organisms.

The fact that 2 fractions of PS I RC were obtained could be due to the use of a higher Triton X-100 concentration in the sucrose gradient, 0.5% vs 0.2% used in purifying *Chlamydomonas, Dunaliella* and spinach complexes [17–20], which was required for complete dissolution of *Prochloron* thylakoids. This might have caused a higher dissociation of chlorophyll molecules from part of the *Prochloron* PS I RC so as to yield the complex with a ratio of chlorophyll/P-700 close to 40, as compared to about 60 chlorophyll *a*/P-700 in similar preparations of higher plants and algae obtained with 0.2% Triton X-100. A ratio of 40 chlorophyll *a*/P-700 was found in higher plants after treatment of PS I RC with SDS [13,20]. However, the possibility that Pa and Pb may represent an in vivo situation of *Prochloron* possessing 2 PS I RCs, one with a larger antenna than the other, cannot be excluded.

Measurements for a chlorophyll *a*/P-700 ratio of 40 have been reported before for the chlorophyll *a*-protein complex of CP-I of *Prochloron* isolated by SDS-PAGE [2]. Using a different method, i.e. solubilization of *Prochloron* thylakoids in SDS and centrifugation on a sucrose density gradient, Hiller and Larkum [6] have isolated a fraction exhibiting a ratio of 87 chlorophyll/P-700 but showing only one polypeptide, by SDS-PAGE, of the same electrophoretic mobility as subunit I reported here, despite the fact that it contained chlorophyll *b* (*a*/*b* ratio of 3.8) and therefore could have also contained the antennae complex of PS I.

With regard to its polypeptide composition, the PS I RC of *Prochloron* was found to be similar to the complex purified from cyanobacteria [18,21] and green algae (fig.2) [19,20]. Like these, it possesses only 4 subunits, unlike the 7 subunits of the complex in higher plants [13,17], although immunological cross-reactivity was found between subunits I and II of *Prochloron* and higher plant complexes. It has been pointed out before that *Prochloron* resembles eukaryotic chloroplasts in several respects [2–6] and cyanobacteria in others [1,7–11]. Comparison of its purified PS I RC complex with those of cyanobacteria, green algae and higher plants presented here demonstrates that the *Prochloron* PS I RC complex indeed resembles the complexes of both cyanobacteria and algae.

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