Photosystem I reaction center from the thermophilic cyanobacterium *Mastigocladus laminosus*

(photosynthesis/immunological crossreactivity/evolution/oxidation–reduction)

R. NECHUSHTAI, P. MUSTER*, A. BINDER*, V. LIVEANU, AND N. NELSON

Department of Biology, Technion–Israel Institute of Technology, Haifa, Israel

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ABSTRACT Photosystem I reaction center was isolated from the cyanobacterium *Mastigocladus laminosus*. It contained four different subunits with molecular masses (as determined by sodium dodecyl sulfate gels) of about 70,000 (subunit I), 16,000 (subunit II), 11,000 (subunit III), and 10,000 (subunit IV) daltons. The purified reaction center contained about 100 chlorophyll *a* molecules per P_{700} ; however, they could be readily depleted down to about 50 chlorophyll *a* per P_{700} without loss in the photochemical activities. The reaction center was active in cytochrome *c* photooxidation, but the photooxidation of an acidic cytochrome, like the *Euglena* cytochrome 552, required the presence of cations. The purified reaction center was found to be similar in several respects to the photosystem I reaction centers from higher plants and, especially, to the one isolated from green algae. Subunit I appeared on sodium dodecyl sulfate gels in the same position and possessed the same shape of an apparent double band as the corresponding subunits I of green plants and of algae. Subunits I and II of photosystem I reaction centers from *Mastigocladus*, higher plants, and green algae showed immunological crossreactivity. This observation might serve as biochemical evidence for the common evolution of the photosystem I reaction centers. In higher plants and green algae subunit II is a product of cytoplasmic ribosomes and therefore, a high degree of homology should have been preserved upon transfer of its gene from the prokaryote to the nucleus of the eukaryotes.

Mastigocladus laminosus is a thermophilic cyanobacterium that grows in alkaline hot springs at temperatures up to 65°C (1, 2). It is able to grow in a wide range of environmental conditions—e.g., from pH 4.5 to pH 9.5 and from 35°C to 65°C. Thermophilic cyanobacteria are the only known organisms having a thermophilic photosynthetic system of the higher plant type. Therefore, it might serve as a convenient source for stable protein complexes, as was demonstrated with other thermophilic bacteria (3). However, during the adaptation to extreme conditions the structure of some protein complexes might change beyond recognition (4). A catalytic sector of proton-ATPase complex was isolated from *Mastigocladus* and was found to resemble the corresponding enzymes in various other organisms (5). To our knowledge, photosystem I reaction center has not been purified heretofore from cyanobacteria and therefore could not be compared with reaction centers that were isolated from higher plants (6–9), green algae (10, 11), and photosynthetic bacteria (12, 13).

In this communication we report on the purification of photosystem I reaction center from the thermophilic cyanobacterium *Mastigocladus laminosus*. The subunit composition and the photobiochemical properties of this reaction center are compared with those of photosystem I reaction centers from green

algae and higher plants. Immunological crossreactivity between subunits I as well as between subunits II from these different sources is indicated and its evolutionary significance is discussed.

MATERIALS AND METHODS

Materials. Most of the chemicals were purchased from Sigma. *Staphylococcus aureus* protein A was obtained from Pharmacia. $Na^{125}I$ was obtained from the Nuclear Research Center, Negev, Israel. Protein A was iodinated with ^{125}I in the presence of chloramine-T. The ^{125}I -labeled protein A (^{125}I -protein A) was stored at –20°C in a medium containing 25 mM acetate/phosphate/NaOH at pH 4.0 and 40% ethanol. DEAE-cellulose (DE-22) was purchased from Whatman and was washed and equilibrated as described (14).

Analytical Methods. Chlorophyll (Chl) was extracted into 80% acetone and measured at 663 nm according to McKinney (15) or total Chl was measured according to Arnon (16). P_{700} was determined as described (6, 7) and protein was assayed according to Lowry *et al.* (17). Gel electrophoresis in slab gels containing an exponential gradient of 10–15% acrylamide was performed according to Douglas and Butow (18). Electrotransfer from slab gels to nitrocellulose paper and immunodecoration by antibodies and ^{125}I -protein A were performed according to Towbin *et al.* (19) with the modifications that were previously described (20, 21). The specific antibodies against denatured membrane proteins were obtained and tested as described (14, 21).

Growing of Cells and Preparation of Membranes. *Mastigocladus laminosus* was obtained from hot springs and grown in medium D of Castenholz (2) at pH 8.2, with constant stirring at 50°C as described (1, 5). The cultures were bubbled with water-saturated air supplemented with 5% CO₂. The cells were illuminated with white light at an intensity of about 10⁵ ergs/cm² per sec (1 erg = 10^{–7} J).

After 4–5 days, each 10-liter culture yielded about 25 g of cells. The cells were washed in a buffer containing 10 mM Tris-HCl (pH 8) and 10 mM NaCl. After centrifugation at 2,000 × *g* for 2 min, the pellet was suspended in 15 ml of the same buffer containing 0.4 M sucrose. The Chl concentration was ≈2 mg/ml and the cells could be stored frozen until used.

The cells were broken twice at about 0°C in a French pressure cell at 20,000 psi (1 psi = 6,895 Pa). The suspension was centrifuged at 2,000 × *g* for 5 min and the pellet was discarded. The supernatant was centrifuged at 150,000 × *g* for 30 min and the resulting pellet was washed as described (10). Finally, the membranes were suspended in a medium containing 50 mM *N*-[tris(hydroxymethyl)methyl]glycine/NaOH (pH 8), 10 mM NaCl, and 0.4 M sucrose, at a Chl concentration of ≈2 mg/ml. The membranes were kept frozen at –70°C.

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Abbreviations: Chl, chlorophyll; kDal, kilodalton(s).

*Present address: Univ. of Zurich, Inst. of Plant Biology, Zollikerstr. 107, Zurich, Switzerland.

RESULTS

Purification of *Mastigocladus* Photosystem I Reaction Center. About 15 ml of membranes was thawed, and octylglucoside (10% solution) and sodium cholate (20% solution) were added to give final concentrations of 1% and 0.5%, respectively. After 20 min of incubation at 0°C, the suspension was centrifuged at $200,000 \times g$ for 60 min. The supernatant contained the proton-ATPase complex and it could be readily purified on sucrose gradients as described (20–22). The pellet was homogenized in a solution containing 25 mM *N*-[tris(hydroxymethyl)methyl]glycine/NaOH (pH 8) and 2% Triton X-100 to give a final Chl concentration of 0.2 mg/ml. After centrifugation at $10,000 \times g$ for 10 min, the supernatant was kept frozen at –20°C until used.

A sample of about 100 ml was thawed and applied to a DEAE-cellulose column (1 × 18 cm). The column then was washed with 100 ml of a solution containing 20 mM Tris·HCl (pH 8) and 2% Triton X-100. The reaction center was eluted with a linear NaCl gradient of 0–300 mM (40 ml in each chamber) in the washing solution. Fractions of 1.6 ml were taken and the fractions from the first green peak of about 100 mM NaCl (containing >0.2 mg of Chl per ml) were pooled and 1-ml portions were applied to linear sucrose gradients of 5–30% in a solution containing 20 mM Tris·HCl (pH 8) and 0.2% Triton X-100. The gradients were centrifuged in a Beckman SW 41 rotor at 35,000 rpm for 18 hr at 2°C. The lower green bands containing the purified reaction center were collected and stored frozen at –70°C. The purified reaction center, like the one isolated from higher plants (6–9) and algae (10, 11), contained about 100 Chl a molecules per P_{700} (Table 1). However, the Chl a could be readily depleted down to about 40–50 molecules per P_{700} by a procedure that was previously described (7, 8, 23). The reaction center was applied on a DEAE-cellulose column (0.8 × 10 cm). The column was washed overnight at 4°C with 250 ml of a medium containing 20 mM Tris·HCl (pH 8) and 2% Triton X-100. The reaction center was eluted by 0.2 M NaCl in the same buffer. During the Chl depletion, the photochemical activities were not affected and the polypeptide composition was not altered.

Fig. 1 shows that the purified photosystem I reaction center is active in cytochrome *c* photooxidation. Inclusion of 10 mM $MgCl_2$ decreased the activity of cytochrome *c* photooxidation. On the other hand, the purified reaction center from *Mastigocladus*, like the one of *Chlamydomonas* (10, 11), was not active in cytochrome 552 photooxidation unless cations such as Mg^{2+} were included in the reaction mixture.

The pH dependency of cytochrome *c* photooxidation is shown in Fig. 2. At low pH values very little activity was obtained. The rate of cytochrome *c* photooxidation increased at higher pH with two pH optima at pH 8 and pH 9.5. The optimum at pH 8 is probably the natural one, whereas at pH values higher than 9.3,

FIG. 1. Photooxidation of cytochrome *c* and cytochrome 552 by *Mastigocladus* photosystem I reaction center. The reaction mixture contained in a final volume of 3 ml: 60 μ mol of *N*-[tris(hydroxymethyl)methyl]glycine/NaOH (pH 8), 50 μ g of horse heart cytochrome *c* or *Euglena* cytochrome 552, 0.3 μ mol of sodium ascorbate, and photosystem I reaction center equivalent to 9.3 μ g of Chl. Light-induced absorbance changes were recorded by an Aminco DW-2a spectrophotometer at 550–535 nm for cytochrome *c* and 552–537 nm for cytochrome 552. The light was provided by a 250-W slide projector through a Schott and Gen cutoff filter (>650 nm). The phototube was protected from the actinic light by a blue filter (Corning 9782). The light intensity was 4×10^4 ergs/cm² per sec at the level of the cuvette. When specified, 30 μ mol of $MgCl_2$ was added to the reaction mixture. L, light; D, dark.

a conformational change in the cytochrome *c* is believed to take place (24).

The effect of temperature on cytochrome *c* and cytochrome 552 photooxidation from *Mastigocladus* and spinach photosystem I reaction centers is depicted in Fig. 3. A temperature optimum of 60°C was measured for the *Mastigocladus* reaction

FIG. 2. The effect of pH on the rate of cytochrome *c* photooxidation by *Mastigocladus* photosystem I reaction center. The reaction mixture contained in a final volume of 1 ml: 50 μ g of cytochrome *c*, 0.1 μ mol of sodium ascorbate, 20 μ mol of 2-(*N*-morpholino)ethanesulfonic acid/*N*-[tris(hydroxymethyl)methyl]glycine/NaOH buffer at the specified pH values, and *Mastigocladus* photosystem I reaction center equivalent to 5 μ g of Chl. Light-induced absorbance changes at 550 nm were recorded by a Cary 118C as described (7).

Table 1. Purification of photosystem I reaction center from *Mastigocladus laminosus*

Purification step	Total Chl, mg	P_{700} , nmol	Chl/ P_{700} , mol/mol	P_{700} recovery, %
Membranes	27.4	27.1	1,126	100
After octyl glucoside and sodium cholate treatment	15.0	26.5	630	98
After DEAE-cellulose column	3.9	17.6	247	65
Purified photosystem I reaction center after sucrose gradient	0.77	7.5	114	28
After Chl depletion	0.22	4.9	50	18

FIG. 3. Effect of temperature on the rate of cytochrome *c* and cytochrome 552 photooxidation by *Mastigocladus* and spinach photosystem I reaction centers. Spinach photosystem I reaction center was purified as described (7, 8). The experimental conditions were as described in the legend to Fig. 1. The temperature was controlled by a water thermostat and it was measured in the cuvette. x—x, Photooxidation of cytochrome 552 by spinach photosystem I reaction center equivalent to 10 μ g of Chl; ○—○, photooxidation of cytochrome 552 by *Mastigocladus* photosystem I reaction center in the presence of 10 mM $MgCl_2$; ●—●, photooxidation of horse heart cytochrome *c* by *Mastigocladus* photosystem I reaction center equivalent to 9.3 μ g of Chl.

center in the rate of photooxidation of both cytochromes. The temperature optimum for the photooxidation of cytochrome 552 by spinach reaction center was 32°C. At 60°C the activity of the spinach reaction center was almost nil.

The polypeptide compositions of *Mastigocladus*, *Chlamydomonas*, *Dunaliella*, and spinach photosystem I reaction centers are shown in Fig. 4. The *Mastigocladus* reaction center resembles in several respects that of the green algae *Chlamydomonas* and *Dunaliella*. They contain four different subunits. In all four species, subunits I appear on NaDodSO₄ gels in a position of 70 kilodaltons (kDa) as an apparent double band. However, this appearance seems to result from a posttranslational modification of a single gene product (unpublished data; ref. 10). The molecular masses of the other subunits are more variable. In calibrated NaDodSO₄ gels, apparent molecular masses of 70, 16, 11, and 10 kDa were obtained for subunits I–IV, respectively, of the *Mastigocladus* reaction center. For the reaction center of *Dunaliella*, molecular masses of 70, 20, 15, and 10 kDa were obtained. Those are quite close to the published molecular masses of *Chlamydomonas* reaction center (10).

Specific antibodies, which were raised against individual subunits of photosystem I reaction centers of *Chlamydomonas* and higher plants, were used to establish immunological cross-reactions among the various polypeptides. Fig. 5 shows that antibody against subunit I of Swiss chard photosystem I reaction center crossreacted with subunits I of reaction centers from spinach, *Chlamydomonas*, and *Mastigocladus*. Similar results were obtained with antibodies against subunits I of spinach and *Chlamydomonas* reaction centers. Subunit I from *Dunaliella* gave positive reaction with these antibodies as well. Fig. 6 depicts a similar experiment with antibody against subunit II of Swiss chard photosystem I reaction center. Subunits II of spinach, *Chlamydomonas*, *Dunaliella* (not shown), and *Mastigocladus* gave pos-

FIG. 4. NaDodSO₄ gel of purified photosystem I reaction centers from spinach, *Chlamydomonas*, *Dunaliella*, and *Mastigocladus*. Spinach photosystem I reaction center was prepared as described (7, 8). *Dunaliella* photosystem I reaction center was purified by a procedure identical to that described for the reaction center from *Chlamydomonas* (10). The preparations were dissociated for about 2 hr at room temperature in the presence of 2% NaDodSO₄/2% 2-mercaptoethanol. Samples containing about 15 μ g of protein were electrophoresed on an exponential 10–15% acrylamide gel in the presence of NaDodSO₄. Lane 1, spinach; lane 2, *Chlamydomonas*; lane 3, *Dunaliella*; and lane 4, *Mastigocladus* photosystem I reaction centers. The various subunits of *Mastigocladus* reaction center are indicated at the left side of the figure.

itive reactions. Similar results were obtained with antibodies against subunits II of spinach and *Chlamydomonas* reaction centers.

DISCUSSION

Cyanobacteria are the only free-living prokaryotes that contain photosynthetic systems of the higher plant type, in which the photochemical events are carried on by two distinct photosystems. Photosystem I, which functions in the reducing side of the electron transport, might have evolved from green photosynthetic bacteria (25). Photosystem II, which functions in the oxidizing side of the chain, might have evolved from a novel invention by using building blocks of yet other systems of photosynthetic bacteria. Cyanobacteria played a central role in the evolution of the system and, therefore, extensive studies have been conducted to elucidate the structure of their photosynthetic system.

Klein and Vernon (26) and Newman and Sherman (27) reported on separation of photosystem I and partial purification of photosystem I reaction center. Their preparations contained multiple polypeptides. We report here on a purification procedure that yielded a photosystem I reaction center from *Mas-*

FIG. 5. Immunological crossreactivity among subunits I of photosystem I reaction centers of plants, algae, and cyanobacteria. Reaction centers of spinach, *Chlamydomonas*, and *Mastigocladus* were electrophoresed on 10–15% acrylamide gel. The protein bands were electrotransferred to nitrocellulose paper and decorated by a specific antibody against subunit I of Swiss chard photosystem I reaction center and ^{125}I -protein A as described (20, 21). Lane 1, spinach photosystem I reaction center equivalent to 0.1 μg of Chl; lane 2, *Chlamydomonas* photosystem I reaction center equivalent to 0.3 μg of Chl; and lane 3, *Mastigocladus* photosystem I reaction center equivalent to 0.4 μg of Chl.

tigocladus laminosus. The purified reaction center was active in cytochrome *c* photooxidation. This reaction was independent of cations and it required alkaline pH values. The purified reaction center also was active in cytochrome 552 photooxidation. Similar to the *Chlamydomonas* reaction center, this reaction was dependent on the presence of cations in the medium (Fig. 1 and refs. 10 and 11). This might present a fundamental difference between the interaction of basic and acidic cytochromes with photosystem I reaction centers. The temperature optimum for the photooxidation of both cytochrome *c* and cytochrome 552 by the *Mastigocladus* reaction center was about 60°C. On the other hand, a temperature optimum of about 32°C was recorded for the spinach reaction center (Fig. 3). This observation showed that the purified reaction center from *Mastigocladus* preserved its temperature stability. All of the photosystem I reaction centers that have been isolated so far contain 100 Chl *a* molecules per P_{700} (6, 7, 9, 10). However, the amount of Chl *a* could be depleted down to about 40 Chl *a* molecules per P_{700} without significant changes in the polypeptide composition and the various activities of the reaction centers (refs. 7, 10, and 23 and Table 1). Also, the stoichiometry of two copies of subunit I in each reaction center seems to be maintained. Therefore, it seems as if the “heart” of photosystem I reaction center has maintained its basic pigment organization throughout evolution from cyanobacteria up to higher plants.

Although subunit I of the various photosystem I reaction centers maintained a strict molecular mass, the other subunits changed positions on the gels and the relations among them is not clear (see ref. 10). A possible common denominator for subunits II of photosystem I reaction centers from eukaryotes is

FIG. 6. Immunological crossreactivity among subunits II of photosystem I reaction center of spinach, *Chlamydomonas*, and *Mastigocladus*. The conditions were as described in the legend to Fig. 5. The nitrocellulose paper was decorated with antibody against subunit II of Swiss chard photosystem I reaction center. Lane 1, spinach; lane 2, *Chlamydomonas*; and lane 3, *Mastigocladus*.

that this subunit was found to be a product of cytoplasmic ribosomes. So far, this has been observed in *Spirodelia* (28), spinach (unpublished data), and *Chlamydomonas* (10). This relation does not hold for prokaryotes such as cyanobacteria. Experiments of immunological crossreactivity are described in Figs. 5 and 6. The technique of electrotransfer and immunodecoration can indicate similarities in structure among polypeptides from various sources. By using this technique, it was found that the β subunits of proton-ATPase complexes crossreacted with the antibodies of each other (20). Similarly, we report here that antibody that was raised against subunit I of Swiss chard photosystem I reaction center crossreacted with reaction centers of higher plants (spinach), green algae (*Chlamydomonas*), and cyanobacterium (*Mastigocladus*). Similar results were obtained with membranes and with antibody against subunit I of *Chlamydomonas* reaction center (not shown). This observation indicated that common amino acid sequences were maintained in subunits I of photosystem I reaction centers from cyanobacteria up to higher plants. The observation also might serve as a biochemical indication for the proposed evolutionary pathway of photosystem I reaction centers from the reaction center of cyanobacteria.

Antibody against subunit II of the Swiss chard photosystem I reaction center interacted with subunits II of higher plants (spinach), green algae (*Chlamydomonas*), and cyanobacteria (*Mastigocladus*) (Fig. 6). Similar results were obtained with antibody against subunit II of *Chlamydomonas* reaction center. Therefore, analogy for subunits II of reaction centers from cyanobacteria up to higher plants was demonstrated. In spinach this subunit is synthesized on cytoplasmic ribosomes as a larger precursor and it is transported into the chloroplasts by vectorial

processing. A similar process is expected to take place in green algae. In cyanobacteria, this subunit should not cross membranes to reach its site of action. This presents an opportunity to study the biogenesis of closely related protein complexes in prokaryote along with eukaryote.

Recent studies have shown a strong tendency in nature to preserve structures in the protein complexes of energy-transducing membranes (29–33). Photosystem I reaction center is a good example of this phenomenon.

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