A single subunit P-700 reaction center of the thermophilic cyanobacterium *Mastigocladus laminosus*

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The isolation and characterization of a single subunit Photosystem I reaction center of the thermophilic cyanobacterium *Mastigocladus laminosus* is described. The subunit isolated in 0.1% SDS is fully active in P-700 bleaching and oxidizes cytochrome *c* in the light. Depending on denaturing conditions, the protein appears on SDS-PAGE in the 70 kDa or 50 kDa region and contains bound chlorophyll in both forms. The 70 kDa form shows all the activities of an intact reaction center.

Photosynthesis *Cyanobacteria* *Reaction center Photosystem I* *Thermophilicity* Mastigocladus

1. INTRODUCTION

The thermophilic cyanobacterium *Mastigocladus laminosus* grows in hot springs and controlled laboratory cultures under a wide range of environmental conditions [1-3]. Thermophilic cyanobacteria are the only organisms with a thermophilic photosynthetic system of the higher plant type. Therefore, they might serve as a more convenient source for more stable pigment-protein complexes than those isolated from plants. Higher stability has been demonstrated with several proteins of other thermophilic bacteria [4].

Previously, we reported on the purification of the PS I reaction center from *M. laminosus* [5]. The subunit composition and some photochemical properties were compared with those of PS I reaction centers from green algae and higher plants.

We describe here the isolation and characterization of subunit I of the PS I reaction center from *M. laminosus* as a fully active reaction center itself. A similar single subunit reaction center has been described from different sources of higher plants [6].

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Abbreviations: PMSF, phenylmethanesulfonyl fluoride; DCPIP, 2,6-dichlorophenolindophenol; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; PS, photosystem

2. MATERIALS AND METHODS

2.1. *Separation of the subunits*

Cells were grown in medium D of [7] at 50°C as in [8]. The membrane preparation as well as the isolation of the multiple subunit reaction center complex were done as in [5]. Purified reaction centers (about 5 mg chlorophyll) were then suspended in 20 mM Tris-HCl (pH 8) at 0.15 mg chlorophyll/ml. The reaction centers were treated for 60 min in 2% SDS at 40°C in the presence of 1 mM PMSF as protease inhibitor, concentration on an XM-100 Amicon membrane to a volume of about 4 ml and then applied to a Sephacryl S-200 column (2.5 × 100 cm), equilibrated with 0.1% SDS. The subunits were eluted with 20 mM Tris-HCl (pH 8) and 0.1% SDS in fractions of 7 ml.

2.2. *Analytical methods*

SDS-PAGE was done as in [9], with the buffer system of [10]. If not stated otherwise samples for SDS-PAGE were treated with 2% SDS for 20 min at 60°C (SDS:protein ratio = 14:1). Chorophyll

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was measured in 80% acetone at 663 nm as in [11]. P-700 was determined as in [12,13] in the presence of 10 mM Tris-HCl (pH 8), 1.7 mM ascorbate and 1.7 μM DCPIP with a UVIKON spectrophotometer. Photooxidation of cytochrome *c* was measured as the light-induced absorbance changes at 550–540 nm with an Aminco DW-2 spectrophotometer in a reaction mixture containing 10 mM Tris-HCl (pH 8), 50 μg horse heart cytochrome *c*, 5 μmol ascorbate and subunit I reaction center equivalent to 10–20 μg chlorophyll. Protein concentration was assayed as in [14] using the modification in [15].

2.3. *Chemicals*

All chemicals were of the best purity available. Octylglucoside was synthesized as in [16].

3. RESULTS

When the multiple subunit reaction center [5] is treated at 60°C with 2% SDS but at an increasing SDS : protein ratio, the largest subunit (subunit I) appears on SDS-PAGE at two different sites: as 70 kDa and 50 kDa bands (fig.1). Thus, these bands may represent different degrees of dissociation of the same peptide. This was confirmed earlier by us in immunological cross-reactivity experiments, i.e. the 70 and 50 kDa bands both reacted with subunit I-specific antibodies from spinach [5]. Yet, a 50 kDa band was not observed in reaction centers of either spinach or green algae. The smaller subunits are very easily detached from subunit I, under the mildest conditions used and before the 70 kDa form dissociates into the 50 kDa form. Hence, it can be excluded that the change of the molecular mass of subunit I is due to the removal of the smaller subunits from subunit I. Furthermore, differences in the appearance of subunit I on SDS-PAGE are observed, when the reaction centers are treated for 20 min with 2% SDS at a constant SDS:protein ratio, but at increasing temperature. At 20°C subunit I appears as a broad undissociated band of high molecular mass, while at 60°C it is dissociated into the 70 and 50 kDa bands. At 100°C finally, the subunit aggregates and does not enter the gel (fig.2B). In such a gel, before staining, chlorophyll is observed to be bound to subunit I in the undissociated form, in the 70 kDa and to a smaller extent in the 50 kDa

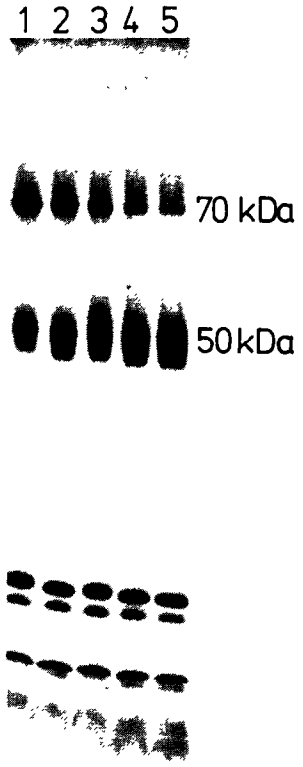

Fig. 1. SDS-PAGE of the multiple subunit reaction center I with increasing SDS:protein ratio during sample preparation. SDS:protein (w/w): 7:1 (1); 14:1 (2); 18:1 (3); 21:1 (4); 28:1 (5). Conditions: 60°C, 20 min and 2% SDS.

form (fig.2A). This again suggests that the chlorophyll-carrying peptide (subunit I) is the same in all 3 bands. Since chlorophyll is bound in all 3 forms, it is also concluded that even under these strong denaturing conditions the peptide is not fully dissociated. At 100°C, when the peptide aggregates, all the chlorophyll is liberated and appears as free pigment at the front.

When the different bands of subunit I are cut out and applied to the glass wall of a cuvette, photobleaching activity can directly be measured in the gel slices. The results show P-700 activity in the 70 kDa band similar to that seen with subunit I isolated by column chromatography, described below (fig.6). However, no reaction center activity can be measured with the 50 kDa gel band.

After treatment of the reaction center complex for 60 min in 2% SDS at 40°C, its subunits can be

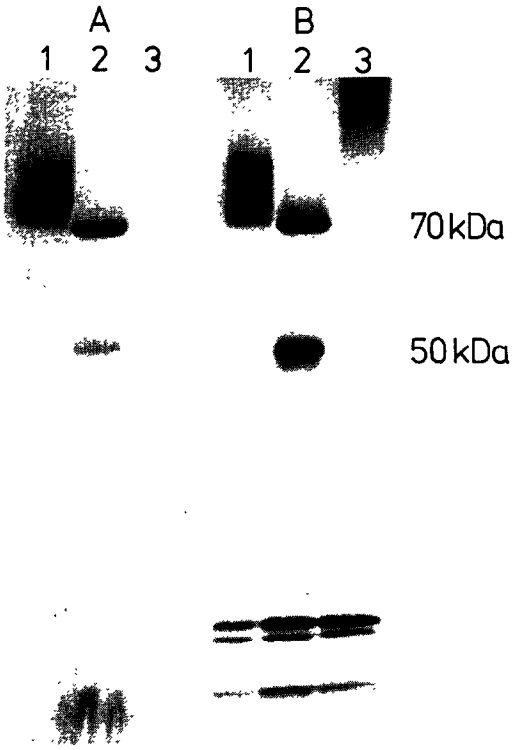

Fig. 2. SDS-PAGE of the multiple subunit reaction center I with varying temperature during sample preparation. (A) Chlorophyll containing bands before staining. (B) After Coomassie blue staining. Conditions: 20 min, 2% SDS and SDS:protein = 14:1; at 20°C (1); 60°C (2) and 100°C (3).

separated preparatively on Sephacryl S-200 in the presence of 0.1% SDS. The elution profile of the column is shown in fig.3. The P-700 activity is eluted in fractions 30–33. The SDS-PAGE in fig. 4 demonstrates that, after treatment with 2% SDS at 60°C for 20 min before application to the gel, these fractions contain the single 50 kDa band of subunit I. However, without further treatment, the same fractions appear as the 70 kDa band only (not shown). These P-700 fractions show spectra which are typical of protein-bound chlorophyll, whereas in fraction 45 free chlorophyll is eluted (fig.5). Fig. 6 shows that the single subunit reaction center (fraction 31) is reversibly bleached at 700 nm and is able to oxidize cytochrome *c* in the light. Subunits II and III in fractions 38–44 are free of chlorophyll and hence do not show any P-700 activity.

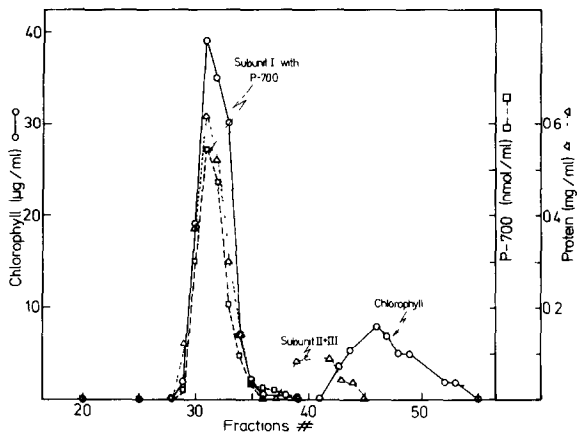

Fig. 3. Elution profile of the Sephacryl S-200 column. Chlorophyll (——), P-700 (– – –), protein (···).

Table 1 summarizes the purification steps and the activities, using reduced cytochrome c as electron donor and oxygen as receptor. The higher chlorophyll:P-700 ratio and the lower cytochrome *c* oxidation activity of subunit I in its 70 kDa form

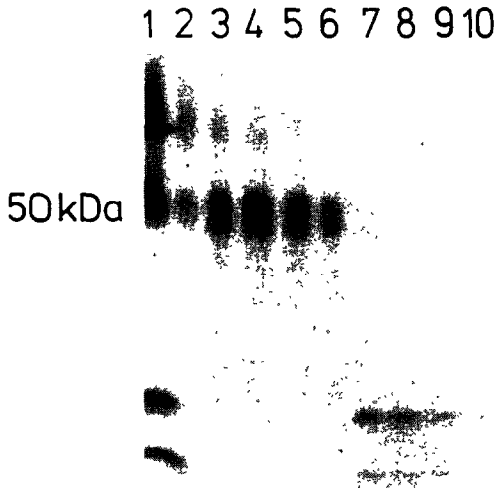

Fig. 4. SDS-PAGE of the fractions of the Sephacryl S-200 column. (1) Sample before column, (2–6) fractions 29–33, (7–10) fractions 40–43.

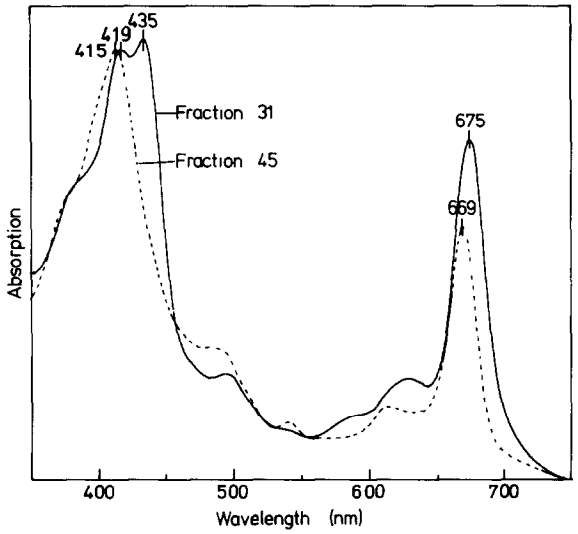

Fig. 5. Absorption spectrum of selected fractions after chromatography on Sephacryl S-200. Fraction 31 (——), fraction 45 (---).

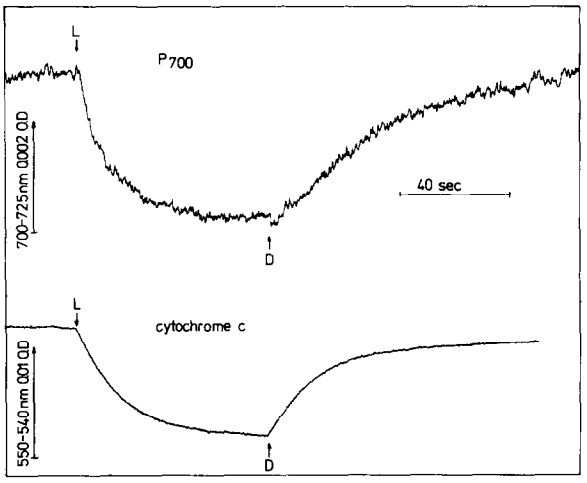

Fig. 6. Photooxidation of P-700 and of cytochrome *c* by the isolated subunit I reaction center. Both measurements were done as described in section 2, but with an Aminco DW-2 spectrophotometer.

are probably due to partial denaturation of the peptide during preparation.

4. DISCUSSION

The different apparent states of the subunit I peptide may be explained by different degrees of dissociation:

(i) In the undissociated form, subunit I is still oligomeric and all the pigments as well as the smaller peptides are attached to the large subunit;

(ii) in the active 70 kDa state, the subunit is monomeric and depleted of the smaller peptides. It still contains most of the pigments, i.e. about 100 chlorophylls per reaction center;

(iii) in the inactive 50 kDa form finally, the peptide has lost most of the chlorophyll, i.e. this form represents roughly the pigment-free peptide chain.

In SDS-PAGE a broadening or splitting of the 70 and 50 kDa bands is often observed. This effect was also reported for PS I reaction centers from higher plants and green algae [6,17]. It has been suggested in [6,17] that subunit I is composed of different but related polypeptides. The same seems to be the case for subunit I described here, i.e. several observations indicate that subunit I consists of two very similar polypeptides.

Despite such similarities, the single subunit I reaction center of PS I from the thermophilic cyanobacterium described here is more resistant to

Table 1

Purification steps and activities of the PS I reaction center in the multiple and single subunit forms

Purification steps	Chl/P-700 ratio (mol/mol)	Cytochrome *c* oxidation (μmol/mg Chl per h)
Whole membranes	1200	–
Triton extract	210	114
After DEAE-cellulose (subunits I–IV)	80	65
After Sephacryl S-200 (subunit I)	100	18

strong detergents and heat compared with any other known reaction center. Furthermore, the cyanobacterial subunit I is smaller than that found in higher plants and green algae [5,6], thus it might represent a phylogenetic older form of the PS I reaction center.

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