PHOTOSYNTHETICA 40 (3): 355-361, 2002

Isolation and characterization of an oxygen evolving photosystem 2 core complex from the thermophilic cyanobacterium *Mastigocladus laminosus*

V.M. RAMESH*, A. FISH, D. MICHAELI, N. KEREN, I. OHAD, L. VORCHOVSKY, and R. NECHUSHTAI

Department of Botany, The Hebrew University of Jerusalem, Givat Ram, Jerusalem, 91904 Israel

Abstract

A novel purification procedure was developed for the isolation of oxygen evolving photosystem 2 (PS2) from *Mastigocladus laminosus*. The isolation procedure involves dodecyl maltoside extraction followed by column chromatography using anion exchange resins. The isolated PS2 reaction center (RC) was analyzed for its biochemical and biophysical characteristics. Analysis by SDS polyacrylamide gel electrophoresis revealed that the complex contained five intrinsic membrane proteins (CP 47, CP 43, D1, D2, and cyt b_{559}) and at least three low molecular mass proteins. The complex exhibited high rates of oxygen evolution [333 mmol(O_2) kg^{-1}(Chl) s^{-1}] in the presence of 2.5 mM 2,6-dimethylbenzoquinone (DMBQ) as an artificial electron acceptor. The red chlorophyll *a* absorption peak of this complex was observed at 673.5±0.2 nm. The isolated PS2 core complex was free of photosystem 1 as inferred from its SDS-PAGE and fluorescence spectrum. The electron transfer properties of the *Mastigocladus* cells and the purified PS2 core complex were further probed by measuring thermoluminescence signals, which indicated the presence of a primary quinone electron acceptor (Q_A) in the purified PS2 core complex.

Additional key words: 2,6-dimethylbenzoquinone; fluorescence; membrane proteins; oxygen evolution rate; reaction center; thermoluminescence.

Introduction

Photosystem 2 (PS2) is a pigment protein complex embedded in the thylakoid membrane of cyanobacteria, algae, and higher plants. It functions as a light-driven water-plastoquinone oxidoreductase (Bricker and Ghanotakis 1996). By utilizing photon energy, PS2 catalyzes the splitting of water into protons, electrons, and molecular oxygen, which is the most strongly oxidizing reaction known to occur in biology (Hankamer *et al.* 1997). Since all of the oxygen in the earth's atmosphere is generated by this energetically extremely unfavorable reaction, it is believed to posses a unique mechanism (Bricker and Ghanotakis 1996). The primary photochemical process takes place in the reaction center (RC) of PS2, which consists of the D1 and D2 protein subunits, cytochrome b_{559} (cyt b_{559}), and the *psb*I gene product (Gounaris *et al.* 1990). The RC proteins are closely associated with two other chlorophyll (Chl) *a* binding proteins [CP47 (*psb*B gene product) and CP43 (*psb*C gene product)], as well as the oxygen evolving complex composed of a four-atom cluster of manganese and a 33 kDa *psb*O extrinsic protein (Ikeuchi and Inoue 1986).

CP47 and CP43 serve as an inner antenna system that is linked to a secondary light-harvesting system. In higher plants and green algae, the Chl *a/b* binding proteins act as the secondary light-harvesting system, while phycobilisomes serve the same purpose in other types of oxygenic photosynthetic organisms, such as red algae and cyanobacteria (Gantt 1996, Bianchetti *et al.* 1998). To elucidate the functional structure of PS2 with the aim of understanding the mechanism of oxygen evolution, a topic of great importance and interest to biologists and biophysicists, a multidisciplinary approach has been undertaken. This includes physical, biochemical, molecular genetics, and crystallographic studies that rely on the isolation of

Received 7 May 2002, *accepted* 25 July 2002.
*Author for correspondence and present address: Department of Plant Biology, P.O. Box 871601, Arizona State University, Tempe, AZ 85287-1601, USA. Fax: (480) 965-6899; e-mail: vmramesh@imap1.asu.edu
Abbreviations: Chl – chlorophyll; DCMU – 3(3,4-dichlorophenyl)-1,1-dimethyl urea; DM – *n*-dodecyl-β-D-maltoside; DMBQ – 2,6-dimethyl-benzoquinone; OEA – oxygen evolution activity; PAGE – polyacrylamide gel electrophoresis; PS – photosystem; Q_A, Q_B – primary and secondary electron acceptor quinones; RC – reaction center; SDS – sodium dodecyl sulfate; TL – thermoluminescence.

pure oxygen evolving PS2 core complex (Debus 1992). Some strains of cyanobacteria can grow and photosynthesize at higher temperatures at which oxygen evolution in higher plants would be completely inactivated.

This suggests that thermophilic cyanobacteria might yield more stable oxygen evolving preparations than those from higher plant chloroplasts (Stewart and Bendall 1980). Several types of oxygen evolving PS2 core complex have been purified from thylakoids of higher plants (MacDonald and Barry 1992, Hankamer *et al.* 1997), green algae (Bumann and Oesterhelt 1994), and cyanobacteria (Noren *et al.* 1991, Kirilovsky *et al.* 1992, Tang and Diner 1994). However, to date, there is no report on the isolation and characterization of pure PS2 core complex from the thermophilic cyanobacterium *Mastigocladus laminosus*. It naturally grows in alkaline hot springs at a temperature of up to 65 °C. Moreover, complexes isolated from this cyanobacterium, such as photosystem 1 (PS1), preserve their thermostability even in isolated form (Nechushtai *et al.* 1983). Hence, isolation of *Mastigocladus* PS2 may result in a very stable PS2 preparation, which will enable the structure determination of this complex.

The present investigation describes the isolation and characterization of the oxygen evolving PS2 core complex from *M. laminosus*. The activity, spectroscopic properties, and polypeptide composition were determined.

Materials and methods

Organism and culture conditions: *Mastigocladus laminosus* was grown in medium D of Castenholz (1969) at pH 8.2 with constant stirring at 50 °C as described by Binder and Bachofen (1979). The cultures were bubbled with water saturated air supplemented with 5 % CO_2. The cells were irradiated with "white light" at an irradiance of about 1 000 J cm^{-2} s^{-1}.

Preparation of thylakoid membranes: Cells grown in 10 000 cm^3 carboys for 3-4 d were allowed to settle and were pelleted by centrifugation at 4 °C for 10 min at 6 000×*g* in a *Sorvall GSA* rotor. The pellets (from 6 carboys ~14 g) were washed once with buffer A [50 mM MES-NaOH, pH 6.0, 20 % glycerol (v/v), 5 mM $CaCl_2$, and 5 mM $MgCl_2$] and pelleted again at 14 000×*g* for 15 min at 4 °C in a *Sorvall GSA* rotor. The cells were resuspended in the same buffer containing a mixture of protease inhibitors, 1 mM phenyl methane sulfonyl fluoride, 1 mM benzamidine, 1 mM amino caproic acid, and incubated for 70 min on an ice-water mixture in the dark. The cells were broken in a Bead-Beater chamber (*Biospec Products*) in the presence of pre-chilled 0.1-mm diameter glass beads. The breakage was carried out using 8 pulses of 20 s each with 5 min cooling intervals. The homogenate was separated from the beads by decantation and the beads were washed 4-5 times with buffer A. Unbroken cells and residual beads were removed from the membrane suspension by centrifugation for 5 min at 3 000×*g* in a *Sorvall GSA* rotor. The thylakoid membranes were then pelleted by centrifugation at 100 000×*g* for 20 min in a *Beckman 50.2 Ti* rotor.

Purification of the thylakoid membrane and isolation of the PS2 core complex: In order to remove the phycobilin proteins present in the thylakoid membrane, the membrane pellets were resuspended in buffer B (buffer A + 5 mM $CaCl_2$) and washed 2 times with the same buffer. The washed thylakoid membranes were resuspended in buffer B at a Chl concentration of 1 kg m^{-3} and stirred gently with 0.1 % (m/v) dodecyl maltoside for about 10 min in the dark and pelleted by centrifugation (184 000×*g* for 30 min in a *Beckman 70 Ti* rotor, 4 °C). The supernatant (containing phycobilin) was discarded and the pellet was resuspended in buffer B at a Chl concentration of 1 kg m^{-3}. A 10 % stock solution of dodecyl maltoside (*Sigma*) was added dropwise to this suspension of thylakoid membranes to give a final concentration of 0.6 % detergent. Extraction proceeded in the dark for 10 min at 0 °C with gentle stirring. The suspension was then centrifuged at 184 000×*g* in a *Beckman 70 Ti* rotor for 30 min at 4 °C. The supernatant was loaded onto a DEAE *Toyopearl 650 S* column (26.0×2.7 cm, a weak anion-exchanger, *Toso Haas*) previously equilibrated with 300 cm^3 of solution A [50 mM Hepes pH 7.2, 10 mM $CaCl_2$, 5 mM $MgCl_2$, 20 % (v/v) glycerol, and 0.03 % dodecyl maltoside] + 20 mM $MgSO_4$. The column was then washed with 900 cm^3 of solution A and then with 680 cm^3 of solution B [50 mM Hepes pH 7.2, 12 mM $CaCl_2$, 5 mM $MgCl_2$, 20 % (v/v) glycerol, and 0.03 % dodecyl maltoside] at a flow rate of 83 mm^3 s^{-1}. To separate the PS2 and PS1 RC complexes, a 2 000 cm^3 of multistep gradient from 5-18 mM $MgSO_4$ in solution B was applied to the column at a flow rate of 66 mm^3 s^{-1}. Fractions with Chl absorbance maxima between 673-674 nm were pooled and immediately used for further purification of PS2.

The pooled PS2 fractions were applied to a *Q-Sepharose-FF* anion exchange column (*Pharmacia*) (1.6×15 cm) that had been equilibrated with solution A. The column was then washed with two column volumes of solution A. To remove the PS1 contaminants, 700 cm^3 of 12 mM $MgSO_4$ in solution B was applied at a flow rate of 133 mm^3 s^{-1}. The PS2 core complex was then eluted by 12-40 mM $MgSO_4$ in solution B at a flow rate of 133 mm^3 s^{-1}. Fractions with Chl absorbance maxima between 673.5 and 673.7 nm were pooled and concentrated to 1-2 kg m^{-3} Chl using an *Amicon 8400* ultrafiltration cell, fitted with *YM-100* membranes and a *Centricon 100* (*Amicon*).

Oxygen evolution was measured with a thermostated Clark type oxygen electrode in the presence of 2.5 mM 2,6-dimethylbenzoquinone (DMBQ) at 40 °C under irradiation with saturated "white light". The activity of the thylakoid membranes and the purified PS2 core complex was measured in the reaction medium containing 50 mM HEPES-NaOH (pH 7.2), 1 M sucrose, 10 mM NaCl, and 20 mM $CaCl_2$. The whole cells were suspended in Castenholz D medium plus 5 mM glucose in the presence of 2 mM DMBQ.

Influence of temperature on O_2 evolution: The samples were suspended in respective solutions [cells in Castenholz D medium and 2.5 mM DMBQ, thylakoids, DM extracts, and purified PS2 core complex in reaction mixture containing 50 mM HEPES-NaOH (pH 7.2), 1 M sucrose, 10 mM NaCl, 20 mM $CaCl_2$, and 2.5 mM DMBQ]. The activity was measured at different temperatures between 20-60 °C.

SDS-PAGE: The polypeptide composition of the PS2 core complex was analyzed by 14 % SDS-PAGE containing 6 M urea as described by Schagger *et al.* (1988). The samples were solubilized in 1 % SDS denaturing buffer by incubation overnight at room temperature. The electrophoresis was performed at 4 °C and stained with *Coomassie Brilliant Blue R-250.*

Optical measurements: Absorbance spectra were recorded on a *Kontron UVIKON 860* spectrophotometer between 300-700 nm. Fluorescence emission spectra were recorded with an *SLM-Aminco 8000* spectrofluorometer. Concentrations of Chl were determined as described by Arnon *et al.* (1974).

Thermoluminescence (TL) was measured for cells and purified PS2 core complex (Zer *et al.* 1994, Inoue 1996, Keren *et al.* 1997). Samples containing 20-40 μg Chl were dark adapted in the home built TL apparatus for 2 min, then excited by continuous irradiation at –40 °C for 1 min. The frozen samples were then heated at 0.6 °C s^{-1} and the photons emitted as a function of temperature were counted. For measuring the TL signal originating from the Q_A^-/S_2 charge recombination, DCMU [3(3,4-dichloro-phenyl)-1,1-dimethyl urea] at a concentration of 2×10^{-6} M was added prior to dark adaptation. In some experiments both DCMU (2×10^{-6} M) and DMBQ (1 mM) were added to the PS2 sample prior to dark adaptation.

Results and discussion

Purification of the intact oxygen evolving PS2 core particles depended on the speed with which the procedure was carried out and the mildness of the extraction and purification. The ratio of PS2 to PS1 in cyanobacteria is ~1 : (5-10) *versus* ~ 1 : 1 in higher plants, which makes the cyanobacteria a less favorable source for the isolation of PS2. However, due to their thermostability, PS2 core particles may exhibit some advantages over higher plants. Therefore, we developed a procedure to isolate PS2 core complex with high purity and activity from *M. laminosus* using ion exchange column chromatography. Fig. 1 indicates schematically the isolation procedure for obtaining the oxygen evolving PS2 core complex.

Fig. 2*A* shows the elution profile using DEAE-*Toyopearl 650 S*, of a dodecyl maltoside extract of the thylakoid membrane. The wash with solution A and solution B elutes successively the free pigments, mainly carotenoids and residual phycobiliproteins. The first peak is a pink-orange color and the following fractions are enriched in phycobiliproteins. Then, $MgSO_4$ eluted the Chl-containing complex. The eluted fractions between 9 and 12 mM $MgSO_4$ showed absorbance to PS2 having a maximal value between 672 and 674 nm. The fractions with absorbance maxima of 673 and 674 nm showed high oxygen evolution activity (OEA) and were pooled for further use. The yield of this purified PS2 complex was approximately 3.53 % of the total starting Chl in the thylakoid membrane (Table 1).

The PS2 fractions obtained from DEAE-*Toyopearl 650 S* column were analyzed by SDS-PAGE and found to contain some PS1 particles (Fig. 4). Therefore, these fractions were again purified by *Q-Sepharose-FF* (strong anion exchanger) column (Fig. 2*B*). The eluted fractions between 12-40 mM $MgSO_4$ in solution B showed typical absorbance to PS2. The fractions with absorbance maxima of 673.5 and 673.7 nm showed high OEA and were pooled and concentrated.

Table 1. Oxygen evolving activities [mmol kg^{-1}(Chl) s^{-1}] in cells, thylakoids, dodecyl maltoside extracts, and PS2 core complex from *Mastigocladus laminosus*. Chl – chlorophyll.

Sample	O_2 evolution	Total Chl [mg]	Yield of total Chl [%]
Cells	81	-	-
Thylakoids	153	65.00	100.00
DM extracts	152	50.00	76.90
PS2 from column I	323	2.30	3.53
PS2 from column II	333	0.42	0.64

Activity and stability of the purified oxygen evolving PS2 core complex: OEA at 40 °C of the cells, thylakoid membranes, dodecyl maltoside extract, and purified PS2 core complex is summarized in Table 1. *Mastigocladus* cells evolved oxygen at a rate of 80 mmol(O_2) kg^{-1}(Chl) s^{-1}, whereas the thylakoid membranes and the dodecyl maltoside extracts practically exhibited an identical OEA of 153 mmol(O_2) kg^{-1}(Chl) s^{-1}. OEAs in purified PS2 core complexes from DEAE-*Toyopearl 650 S* column and from *Q-Sepharose-FF* column were 323 and 333

Growth of *Mastigocladus laminosus* in 10 000 cm^3 carboys (bubbled with air supplemented with 5 % CO_2 and irradiated with continuous "white light")

↓

Breakage of cells using glass beads, 8 × 20 s at 4 °C with 5 min cooling intervals in buffer A (50 mM MES-NaOH, pH 6.0, 5 mM $CaCl_2$, 5 mM $MgCl_2$ and 20 % glycerol)

↓

Isolation of thylakoid membranes by differential centrifugation and washing with buffer B (buffer A + 5 mM $CaCl_2$)

↓

Wash of thylakoid membrane, 2 times with buffer B (buffer A + 5 mM $CaCl_2$)

↓

Wash of thylakoid membrane with 0.1 % dodecyl maltoside

↓

Detergent extraction of proteins from thylakoid membrane with 0.6 % dodecyl maltoside, extraction proceeded for 10 min on ice with gentle stirring

↓

Purification of PS2 by ion exchange column chromatography (*Deae-Toyopearl 650 S* and *Q-Sepharose-FF* column)

↓

Analysis of purified PS2 core complex by SDS-PAGE, native PAGE

Fig. 1. Flow diagram of the preparation of thylakoid membranes and purification of oxygen evolving PS2 core complexes from *Mastigocladus laminosus*

mmol(O_2) kg^{-1}(Chl) s^{-1}, respectively, showing an approximate 2.1-fold increase of OEA compared to the thylakoids.

OEA of the purified PS2 complex was stable. Almost no activity loss was observed upon 24 h storing of the concentrated complex at 4 °C in the dark. Rapid freezing and thawing of the concentrated complex in liquid nitrogen also did not inactivate OEA. The PS2 core complex from *M. laminosus* is more stable than the PS2 isolated from *Synechocystis* PCC 6803 (Tang and Diner 1994).

Influence of temperature on oxygen evolution: *Mastigocladus* cells, thylakoids, dodecyl maltoside extracts, and purified PS2 core complex were analyzed for their OEA at different temperatures from 20-60 °C (Fig. 3). *Mastigocladus* cells showed maximum activity [161 mmol(O_2) kg^{-1}(Chl) s^{-1}] at 55 °C, whereas cell free preparations such as thylakoids, dodecyl maltoside extracts, and the purified PS2 core complex exhibited maximum activity [153, 152, and 333 mmol(O_2) kg^{-1}(Chl) s^{-1}, respectively] at 40 °C and they could not stay above 45 °C. At higher temperatures, particularly at 50 and 55 °C, a marked reduction in activity was observed. The reason for the shift in OEA from 55 °C (optimum temperature for cells) to 40 °C (optimum temperature for thylakoids, DM extracts, and PS2) is not known. However, similar phenomenon was reported from the thermophilic cyanobacterium *Phormidium laminosum* (Stewart and Bendall 1980). The thermal stability of OEA in growing cultures of *P. laminosum* was not retained when the cells were broken to yield membrane fragments (Stewart and Bendall 1980). Furthermore, the purified oxygen evolving PS2 core complex (by DEAE *650 M* column) from the

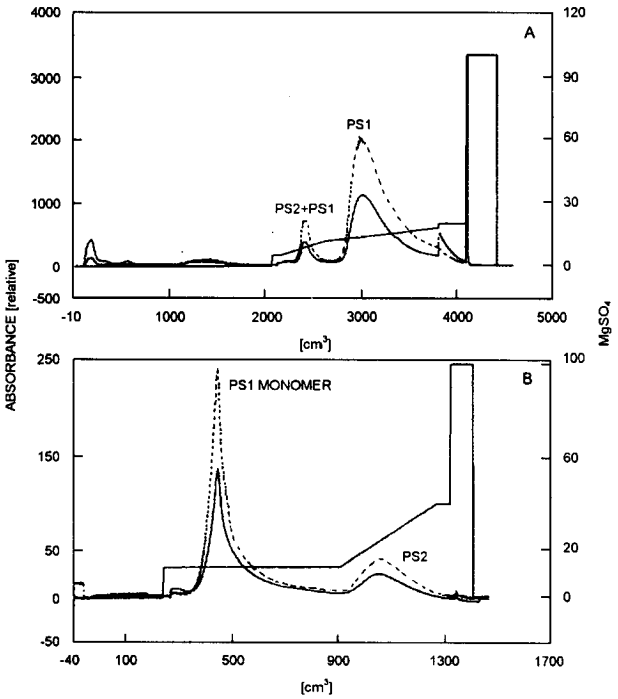

Fig. 2. Elution profile of the dodecyl maltoside extract on a DEAE-*Toyopearl 650 S* column (*A*) and further purification of PS2 fractions on a *Q-Sepharose-FF* column (*B*). Absorbance was measured at the red absorption maximum 673 nm (*dashed line*) and at 280 nm (*solid line*)

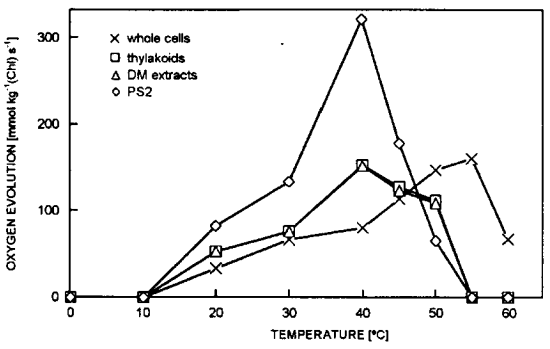

Fig. 3. Influence of temperature on oxygen evolution in cells, thylakoids, DM extracts, and in PS2 core complex.

thermophilic cyanobacterium *Synechococcus vulcans* (Koike *et al.* 1989) and from the *Synechococcus* sp. (Oren-Shamir *et al.* 1995) exhibited maximum OEA at 40 °C. However, OEA of purified PS2 core complex from *Synechococcus* sp. was lower [83-111 mmol(O_2) kg^{-1}(Chl) s^{-1}] than that of the crude preparation [278 mmol(O_2) kg^{-1}(Chl) s^{-1}] (Oren-Shamir *et al.* 1995).

Polypeptide composition: SDS-PAGE analysis of polypeptide composition of the purified oxygen evolving PS2 core complex revealed a high enrichment in its complex from this preparation (Fig. 4). The purified PS2 core complex consists of subunits with apparent molecular masses of ~ 47, 43, 33, 30, 28, 22, 18, 9.5, 6.1, 4.3 and 3.8 kDa. The bands at 47 and 43 are CP 47 apoprotein (*psbB* gene product) and CP 43 apoprotein (*psb*C gene product). The 33 kDa subunit may be the manganese stabilizing protein (*psb*O gene product). The bands at 30 and 28 kDa are D2 (*psb*D gene product) and D1 (*psb*A gene product) proteins, respectively. The 9.5 kDa subunit corresponds to α (*psb*E gene product) subunit of cytochrome b_{559}. There are at least 3 additional bands of lower molecular mass, possibly corresponding to the products of *psb*M (3.8 kDa), *psb*K (3.9 kDa), and *psb*H (6.5 kDa). The band at 22 kDa may be the *psb*S gene product and the band near 18 kDa was not identified.

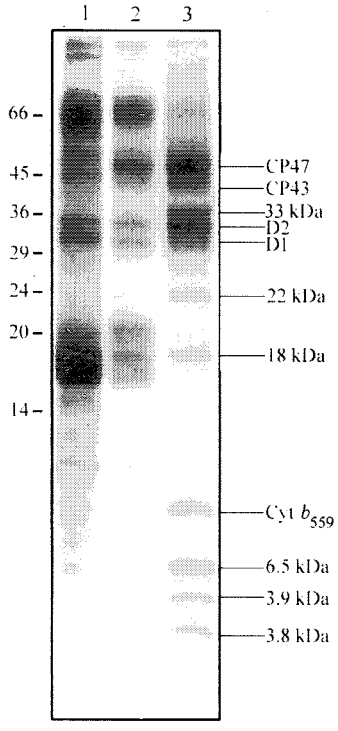

Fig. 4. SDS-PAGE of the purified PS2 core complex from *Mastigocladus laminosus*. Lane *1*: Thylakoid membrane, lane *2*: PS2 core complex fraction after purification by DEAE-*Toyopearl 650 S* anion exchange column, and lane *3*: PS2 core complex after purification on the *Q-Sepharose* anion exchange column.

However, that band was reported to be present in the PS2 RC preparation from the cyanobacterium *Synechocystis* sp. 6803 (Rögner *et al.* 1990). This is a typical polypeptide composition of the oxygen evolving PS2 core complex and is similar to the one isolated by anion exchange column chromatography (DEAE-*Toyopearl 650 M*) from the thermophilic cyanobacterium *S. vulcans* (Koike *et al.* 1989). SDS-PAGE analysis of the oxygen evolving PS2 from the *S. vulcans* revealed that the complex contained at least 7 low molecular mass proteins in addition to the well characterized [CP 47, CP 43 apoproteins, 33 kDa (*psb*O gene product) extrinsic protein, D1, D2 proteins, and the large subunit of cytochrome b_{559}]. Similar pattern of polypeptide composition was also observed in the purified PS2 core complex from the cyanobacterium *Synechocystis* sp. (Tang and Diner 1994). However, only 6 polypeptide subunits (CP 47, CP 43, D1, D2, 38 kDa subunit, and α-subunit of cytochrome b_{559}) showed similarity with the previously reported polypeptide composition of *Synechocystis sp.* (Tang and Diner 1994).

Absorbance and fluorescence spectra: The room temperature absorption spectrum of the purified oxygen-evolving PS2 core complex is illustrated in Fig. 5. The

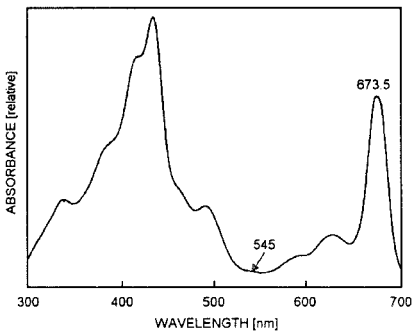

Fig. 5. Absorption spectrum of the purified PS2 core complex from *Mastigocladus laminosus* recorded at room temperature.

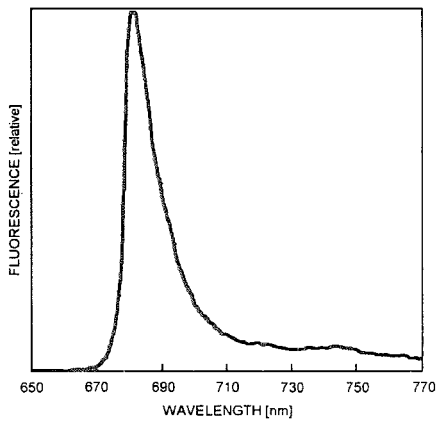

Fig. 6. Fluorescence emission spectrum of the PS2 core complex from *Mastigocladus laminosus* at 77 K.

red Chl *a* absorption peak of this complex was observed

at 673.5±0.2 nm. This is a typical absorbance spectrum of a PS2 core complex, nearly identical to the oxygen evolving PS2 core complex purified from the cyanobacteria *Synechocystis* sp. (Rögner *et al.* 1990, Tang and Diner 1994) and *Synechococcus* sp. (Oren-Shamir *et al.* 1995) and from spinach (Tang *et al.* 1990).

The spectra indicate that phycobilisome pigments, typically absorbing between 600 and 650 nm, were completely removed from the complex. Also a large fraction of the carotenoids, absorbing between 450 and 500 nm, was removed from the preparations. In the PS2 spectrum, the presence of a small peak near 545 nm, ascribed to pheophytin *a*, is also evident.

The fluorescence emission spectrum of isolated PS2 core complex (Fig. 6) shows the emission peak of PS2 complex at 689 nm and negligible fluorescences at 720 nm (PS1 antenna complex) and at 705 and 735 nm (PS1 RC core antenna) (Rögner *et al.* 1990).

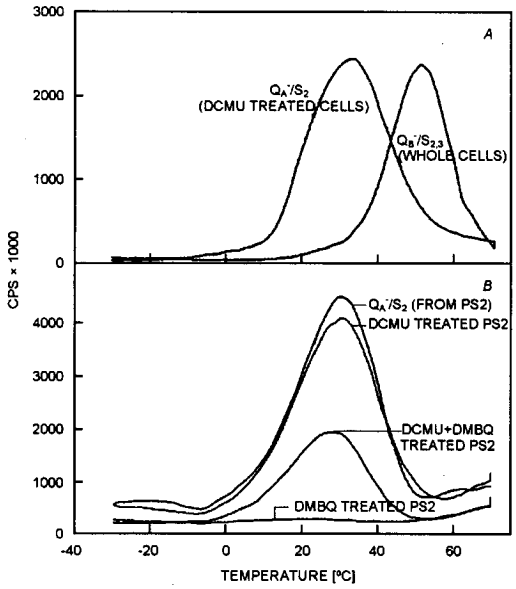

Fig. 7. Thermoluminescence of *Mastigocladus* cells (*A*) and purified PS2 core complex (*B*) measured either with DCMU alone or with combinations of DCMU and DMBQ. For further details see Materials and methods.

TL analysis: The electron transfer properties of the *Mastigocladus* cells and the purified PS2 core complex were further probed by measuring thermoluminescence signals (Fig. 7*A*). The whole cells emitted a TL signal around 50 °C. The signal emitted around 50 °C may be due to the charge recombination between Q_B^-/S_2, S_3 state of the water oxidizing complex. When electron transfer between Q_A and Q_B was blocked by 2×10^{-6} M DCMU prior to the excitation, the *Mastigocladus* cells emitted a TL signal with a maximum around 30 °C that may arise from the charge recombination between Q_A^- and S_2. The purified PS2 core complex (Fig. 7*B*) also registered a band around 30 °C that may also arise from the charge recombination between the Q_A^-/S_2 state. These results clearly indicate the presence of primary quinone electron acceptor (Q_A) in the purified PS2 core complex. There was no change in the TL signal when the PS2 was treated with DCMU and DCMU + 1 mM DMBQ combinations but the TL intensity was sharply reduced in DCMU + DMBQ treatment. The fact that this TL signal was completely inhibited when the purified PS2 core complex was treated with 1 mM DMBQ also indicates that the electrons can be transferred to DMBQ and that the Q_A site in purified PS2 core complex was not affected.

In the present investigation, we have described a novel method to isolate oxygen evolving PS2 core complexes from the thermophilic cyanobacterium *M. laminosus*. Spectroscopic and TL characterization of the preparation indicate that the isolated complex is highly purified and fully intact with regard to PS2 electron transfer until Q_A. The purified complex also preserved the high OEA [333 mmol kg(Chl) s^{-1}]. This method can be useful to isolate highly purified and fully intact PS2 core complex from any thermophilic cyanobacterium.

References

Arnon, D.I., McSwain, B.D., Tsujimoto, H.Y., Wada, K.: Photochemical activity and components of membrane preparations from blue-green algae. I. Coexistence of two photosystems in relation to chlorophyll *a* and removal of phycocyanin. – Biochim. biophys. Acta **357**: 231-245, 1974.

Bianchetti, M., Zheleva, D., Deak, Z., Zharmuhamedov, S., Klimov, V., Nugent, J., Vass, I., Barber, J.: Comparison of the functional properties of the monomeric and dimeric forms of the isolated CP47-reaction center complex. – J. biol. Chem. **273**: 16128-16133, 1998.

Binder, A., Bachofen, R.: Isolation and characterization of a coupling factor I ATPase of the thermophilic blue-green alga (cyanobacterium) *Mastigocladus laminosus*. – FEBS Lett. **104**: 66-70, 1979.

Bricker, T.M., Ghanotakis, D.F.: Introduction to oxygen evolution and the oxygen-evolving complex. – In: Ort, D.R., Yocum, C.F. (ed.): Oxygenic Photosynthesis: The Light Reactions. Pp. 113-136. Kluwer Academic Publishers, Dordrecht – Boston – London 1996.

Bumann, D., Oesterhelt, D.: Purification and characterization of oxygen-evolving photosystem II core complexes from the green alga *Chlamydomonas reinhardtii*. – Biochemistry **33**: 10906-10910, 1994.

Castenholz, R.W.: Thermophilic blue-green algae and the thermal environment. – Bacteriol. Rev. **33**: 476-504, 1969.

Debus, R.J.: The manganese and calcium ions of photosynthetic oxygen evolution. – Biochim. biophys. Acta **1102**: 269-352, 1992.

Gantt, E.: Pigment protein complexes and the concept of the photosynthetic unit: Chlorophyll complexes and phycobilisomes. – Photosynth. Res. **48**: 47-53, 1996.

Gounaris, K., Chapman, D.J., Booth, P., Crystall, B., Giorgi, L.B., Klug, D.R., Porter, G., Barber, J.: Comparison of the D1/D2 cytochrome *b*-559 reaction centre complex of photo-

system two isolated by two different methods. – FEBS Lett. **265**: 88-92, 1990.

Hankamer, B., Nield, J., Zheleva, D., Boekema, E., Jansson, S., Barber, J.: Isolation and biochemical characterisation of monomeric and dimeric photosystem II complexes from spinach and their relevance to the organization of photosystem II *in vivo*. – Eur. J. Biochem. **243**: 422-429, 1997.

Ikeuchi, M., Inoue, Y.: Characterization of O_2 evolution by a wheat photosystem II reaction center complex isolated by a simplified method: disjunction of secondary acceptor quinone and enhanced Ca^{2+} demand. – Arch. Biochem. Biophys. **247**: 97-107, 1986.

Inoue, Y.: Photosynthetic thermoluminescence as a simple probe of photosystem II electron transport. – In: Amesz, J., Hoff, A.J. (ed.): Biophysical Techniques in Photosynthesis. Pp. 93-107. Kluwer Academic Publishers, Dordrecht – Boston – London 1996.

Keren, N., Berg, A., van Kan, P.J., Levanon, H., Ohad, I.: Mechanism of photosystem II photoinactivation and D1 protein degradation at low light: The role of back electron flow. – Proc. nat. Acad. Sci. USA **94**: 1579-1584, 1997.

Kirilovsky, D.L., Boussac, A.G.P., Van Mieghem, F.J.E., Ducruet, J.-M.R.C., Sétif, P.R., Yu, J., Vermaas, W.F.J., Rutherfold, A.W.: Oxygen-evolving photosystem II preparation from wild type and photosystem II mutants of *Synechocystis* sp. PCC 6803. – Biochemistry **31**: 2099-2107, 1992.

Koike, H., Mamada, K., Ikeuchi, M., Inoue, Y.: Low-molecular-mass proteins in cyanobacterial photosystem II: identification of *psb*H and *psb*K gene products by N-terminal sequencing. – FEBS Lett. **244**: 391-396, 1989.

MacDonald, G.M., Barry, B.A.: Difference FT-IR study of a novel biochemical preparation of photosystem II. – Biochemistry **31**: 9848-9856, 1992.

Nechushtai, R., Muster, P., Binder, A., Liveanu, V., Nelson, N.: Photosystem I reaction center from the thermophilic cyanobacterium *Mastigocladus laminosus*. – Proc. nat. Acad. Sci. USA **80**: 1179-1183, 1983.

Noren, G.H., Boerner, R.J., Barry, B.A.: EPR characterization of an oxygen-evolving photosystem-II preparation from the transformable cyanobacterium *Synechocystis* 6803. – Biochemistry **30**: 3943-3950, 1991.

Oren-Shamir, M., Sai, P.S.M., Edelman, M., Scherz, A.: Isolation and spectroscopic characterization of a plantlike photosystem II reaction center from the cyanobacterium *Synechocystis sp.* 6803. – Biochemistry **34**: 5523-5526, 1995.

Rögner, M., Nixon, P.J., Diner, B.A.: Purification and characterization of Photosystem I and Photosystem II core complexes from wild-type and phycocyanin-deficient strains of the cyanobacterium *Synechocystis* PCC 6803. – J. biol. Chem. **265**: 6189-6196, 1990.

Schagger, H., Aquila, H., Jagow, G. von: Coomassie blue-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for direct visualization of polypeptides during electrophoresis. – Anal. Biochem. **173**: 201-205, 1988.

Stewart, A.C., Bendall, D.S.: Photosynthetic electron transport in a cell-free preparation from the thermophilic blue-green alga *Phormidium laminosum*. – Biochem. J. **188**: 351-361, 1980.

Tang, X.-S., Diner, B.A.: Biochemical and spectroscopic characterization of a new oxygen-evolving photosystem II core complex from the cyanobacterium *Synechocystis* PCC 6803. – Biochemistry **33**: 4594-4603, 1994.

Tang, X.-S., Fushimi, K., Satoh, K.: D1-D2 complex of the photosystem II reaction center from spinach. Isolation and partial characterization. – FEBS Lett. **273**: 257-260, 1990.

Zer, H., Prasil, O., Ohad, I.: Role of plastoquinol oxidoreduction in regulation of photochemical reaction center II D1 protein turnover *in vivo*. – J. biol. Chem. **269**: 17670-17676, 1994.