Biochimica et Biophysica Acta 932 (1988) 258–267
Elsevier

BBA 42711

Preparation and characterization of Photosystem II particles from a thermophilic cyanobacterium

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(Received 27 August 1987)
(Revised manuscript received 3 November 1987)

Key words: Oxygen evolution; Photosystem II; P-680$^+$ reduction; (*M. laminosus*)

Photosystem II particles have been isolated and purified from the thermophilic cyanobacterium *Mastigocladus laminosus* after sulfobetaine treatment. The light-induced steady-state electron transport, flash-induced absorption changes at 830 nm and 325 nm, reflecting the turnover of P-680 and of the acceptor side, respectively, have been measured. In the presence of artificial electron acceptors the preparations showed high O_2-evolution activities (e.g., 3000–4000 μmol O_2 per mg Chl *a* per h with phenyl-*p*-benzoquinone) which were DCMU-sensitive. O_2-evolution activity was stable in the presence of various cations (10 mM) and of glycerol (25% (v/v)) in the suspending medium. Furthermore, the activity was stable up to 60 °C and maximum rate of the electron transport was observed at 50 °C. The spectrum of the particles showed a chlorophyll *a* maximum at approx. 673 nm, some carotenoids were still present but phycocyanin was completely removed. After SDS-polyacrylamide gel electrophoresis the preparations contained mainly nine protein bands at 110, 105, 60, 55, 35, 33, 22, 18 and 17 kDa. Under repetitive laser flash excitation the P-680$^+$-reduction kinetics contained components in the nanosecond and microsecond range with an absorption rate of approx. 1:1. When PS-II particles were purified at room temperature by anion-exchange chromatography nanosecond relaxation kinetics were observed even though the particles did not evolve oxygen. The gel analysis for this preparation showed mainly four protein bands at 60, 55, 35 and 18 kDa.

Introduction

Photosynthetic water oxidation takes place in Photosystem II via a four-step univalent redox reaction sequence. The kinetic pattern of the process has been resolved in detail whereas fundamental questions about the mechanism and the structural organization of the complex still remain to be answered (for a recent review see Ref. 1). One way to attack the latter problem leads to the isolation and separation of Photosystem II from the other components of the thylakoid membrane and the functional analysis of these samples, con-

Abbreviations: Chl, chlorophyll; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-propanesulfonate; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; DCIP, 2,6-dichlorophenolindophenol; DIDS, diisothiocyanostilbene-2,2′-disulfonic acid; FeCN, $K_3Fe(CN)_6$; FWHM, full width at half maximum; LDAO, *N*,*N*-dimethyldodecylamine *N*-oxide; OG, octyl-β-D-glucopyranoside; P-680, primary electron donor of Photosystem II; PBQ, phenyl-*p*-benzoquinone; PS II, Photosystem II; Q_A and Q_B, primary and secondary plastoquinone of Photosystem II, respectively (electron acceptors); SB-12, sulfobetaine 12 (*N*-dodecyl-*N*,*N*-dimethylammonio-3-propanesulfonate); TEA, triethanolamine hydrochloride.

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taining only a fraction of the complete energy-transducing system. However, the procedures developed for the isolation of membrane protein complexes are often too harsh to retain the structural and functional integrity of the Photosystem II. Especially the oxygen-evolving capacity was shown to be highly sensitive to treatment with different types of detergent [2,18].

In recent years cyanobacteria were found to be an excellent source of material for the isolation of membrane fragments retaining high rates of oxygen evolution. Such cyanobacterial preparations have been reported for the mesophilic *Phormidium laminosum* [3] and the thermophilic *Synechococcus* sp. [4]. Based on previous experience with the isolation of other photosynthetic complexes [5] we chose to use the thermophilic cyanobacterium *Mastigocladus laminosus* for the preparation of PS-II particles, an organism which grows optimally at temperatures up to 65 °C [6] and with the goal on one side to isolate PS-II membrane fragments which retain a high capacity for oxygen evolution, and on the other, to obtain a photochemically active preparation with the minimum number of protein subunits. In this communication such highly active oxygen-evolving PS-II particles are characterized by measurements of the turnover of the photoactive chlorophyll *a*, P-680, and of redox components on the donor and acceptor side of PS-II, respectively. Furthermore, a preparation consisting of four major polypeptides active in a limited photosynthetic electron transport is described.

Material and Methods

The thermophilic cyanobacterium *M. laminosus* [7] was grown in 10-l flasks in the inorganic medium D of Castenholz [8] at 50 °C (pH 7). The culture was gassed with air (2 l/min) enriched with 5% (v/v) CO_2 and illuminated with fluorescent lamps (Silvania lifeline, 25 W, 15 W/m^2). The cells were harvested in the late log phase by centrifugation in a Westfalia separator and washed with distilled water. For further use the cells were frozen in liquid N_2 and stored at −80 °C. For preparation of the membranes the cells were resuspended in 30 mM TEA (pH 7), 10 mM $MgCl_2$, 25% (v/v) glycerol (buffer 1) at a concentration of 2–3 mg Chl/ml and broken with a French press (Aminco). The cells were passed through twice at a pressure of 39 MPa and the suspension centrifuged at 600 × *g* for 5 min. The pellet of unbroken cells and cell debris was discarded and the supernatant recentrifuged at 100 000 × *g* for 60 min. To remove the phycocyanin the membranes were washed with 30 mM TEA (pH 7), 10 mM $MgCl_2$ (buffer 2). Membranes were then resuspended in buffer 1 to a concentration of 2–3 mg Chl/ml and stored at −80 °C until use. A temperature of 4 °C was maintained throughout the preparation.

For solubilisation of the PS-II the membranes were diluted to 1 mg Chl/ml with buffer 1 plus 0.35% (w/w) detergent SB-12, giving a detergent to Chl *a* ratio of 3.5 : 1 (w/w) according to the method described in Refs. 3 and 4. The mixture was incubated for 20 min in the dark at 25 °C and then centrifuged at 100 000 × *g* for 60 min. The resulting supernatant which contained the accessory pigments and the PS-II particles was passed through a Sepharose 6B column (2.5 × 50 cm) equilibrated with buffer 1 lacking the detergent. This caused an aggregation of the PS-II particles leading to elution with the void volume. In other experiments the extract was adsorbed on an anion exchange column (Mono Q, Pharmacia) at room temperature from which the particles were eluted with a gradient of 0.01–0.5 M $MgCl_2$ in buffer 1 that contained 0.15% (w/w) SB-12. Oxygen evolution was measured with a Clark-type oxygen electrode (Rank brothers). The reaction vessel was kept at 25 °C unless stated otherwise. Saturating illumination was provided from a video lamp (1000 W, Osram). The light beam was passed through 5 cm of water as a heat shield. The reaction mixture contained 3 ml buffer 2, 10 μg Chl a plus 1.5 mM $K_3Fe(CN)_6$ or 0.3 mM phenyl-*p*-benzoquinone. Chlorophyll *a* concentration was determined by the method of Arnon [9]. The concentration of cytochrome *b*-559 was calculated according to Ref. 10. SDS-polyacrylamide gel electrophoresis analysis was carried out using a 5% stacking gel and a 12% polyacrylamide running slab gel [11]. The samples were incubated with 2% SDS and 2% 2-mercaptoethanol for 1 min at 100 °C before loading on the gel.

Absorption spectra were measured on a Uvikon 810 spectrophotometer (Kontron). The number of

oxygen evolving PS-II reaction centers per Chl was determined by measuring the average O_2 yield in repetitive single turnover flashes, averaging 120 flashes with a frequency of 2 Hz [12]. Laser-flash-induced absorption changes at 830 nm [13,14] and at 325 nm [15] were monitored at room temperature in a sample containing buffer 1, 1 mM $K_3Fe(CN)_6$, 80 μg Chl/ml and 10 μg Chl/ml, respectively.

Results

Isolation procedure and characterization of the preparations

Basically a three-step procedure was developed: (a) preparation of active membranes; (b) detergent solubilization of these membranes; and (c) isolation and purification of PS-II particles. Cells frozen in liquid N_2 have been stored for several months without loss of activity while the oxygen-evolving capacity disappeared completely after a few months at −20°C. Thylakoid membranes prepared by French-press treatment were essentially free of the accessory pigment phycocyanin after centrifugation. Depending on the physiological state of the cells these membrane preparations showed typical O_2 evolution activities of 300–500 μmol O_2 per mg Chl per h with $K_3Fe(CN)_6$ as electron acceptor. The isolation of highly active PS-II fragments is critically dependent on the type of detergent and the solubilization conditions used. Several detergents such as Triton X-100, LDAO, deriphat, CHAPS and cholat have been tested and proved to be unsuitable, since they either destoryed the PS-II activity (Triton X-100, LDAO) or did not lead to successful solubilization of the membranes (deriphat, CHAPS, cholat).

The detergent sulfobetaine (SB-12) at concentrations around 0.35% (w/w) was found to provide optimal conditions for solubilization of the PS-II of *M. laminosus* with high rates of oxygen evolution. With this detergent treatment the small PS-II particles (equivalent to about 10% of the total chlorophyll) were removed from the membrane matrix which still contained most of the PS-I activity. A detergent-to-chlorophyll ratio of 3.5 as suggested by Ref. 3 was usually optimal. Higher detergent concentrations led to a solubilization of both the PS-II and the PS-I, concomitant with a decrease in oxygen evolving capacity. Besides SB-12, octyl-β-D-glucoside, another mild detergent, was also effective in solubilizing active particles, the optimal concentration was around 0.55% (w/w).

This one step treatment with SB-12 resulted in a supernatant which exhibits a 3–4-fold increase in O_2-evolution activity on a chlorophyll basis compared to the original membranes (Table I). Rates up to 5500 μmol O_2 per mg Chl per h were obtained when phenyl-*p*-benzoquinone served as electron acceptor at 25°C. The high activity in the presence of phenyl-*p*-benzoquinone and its sensitivity to DCMU (vide infra) are indicative of a high degree of structural integrity of the Q_B-binding site. This supports the previous findings showing that after mild trypsin treatment of thylakoids the endogenous plastoquinone as well as added quinones lost their electron acceptor capacity, whereas $K_3Fe(CN)_6$ mediated a PS-II electron transport which was rather DCMU insensitive [16,17].

The presence of 25% (v/v) glycerol in all steps of the preparation was found to be necessary to stabilize the O_2-evolution capacity in the presence of detergents, an observation made earlier by Ref. 18. The electron transport from water to $K_3Fe(CN)_6$ or PBQ of our PS-II-enriched preparation was fully (100%) sensitive to 5 μM DCMU or 3 mM NH_2OH, indicating that it was a true PS-II reaction. When the crude extract was passed through Sepharose 6B equilibrated with buffer lacking the detergent the particles aggregated and separated from the more slowly eluting

TABLE I

O_2-EVOLUTION ACTIVITY OF PHOTOSYSTEM II DURING ISOLATION

The measurements were done as described in Material and Methods.

Preparation	Acceptor (μmol O_2 per mg Chl per h)	
	FeCN	PBQ
Membranes	300– 500	800–1100
Supernatant	1000–1400	3000–4000
Purified with Sepharose 6B	1000–1300	3500–4000

phycocyanin. The allophycocyanin which is tightly bound to the reaction center II was not always removed completely from the PS-II particles. A successful purification seems to be dependent on the physiological state of the cells. The particles obtained after gelfiltration, showing high oxygen evolution rates, were defined as oxygen evolving particles.

For the determination of cytochrome *b*-559 content spectra between 530 and 580 nm were taken [10]. The Chl *a*/cytochrome *b*-559 ratio was about 30, and about half of the cytochrome became reduced by hydroquinone. All cytochrome *b* was reduced by dithionite, suggesting that there were equal amounts of the high-potential and the low-potential forms.

Particles purified with an anion-exchange column such as Mono Q (at room temperature) were free from the contaminants phycocyanin and allophycocyanin, and furthermore part of the carotenoids were removed. This PS-II preparation has lost the ability to evolve oxygen; henceforth, these particles are referred to as non-oxygen-evolving particles. These particles retained their spectrum and still carried out a light-induced electron transport from diphenylcarbazide to DCIP (not shown). The absorption spectra depicted in Fig. 1 show that the main absorption of membranes is due to Chl *a*. The extract exhibits a peak at 650 nm, indicating a contamination with phycocyanin. Compared with the original membranes the absorption maximum of the oxygen evolving particles is slightly shifted towards shorter wavelength (from 678 nm to 673 nm) and a shoulder at 485 nm that derives from the carotenoids becomes prominent.

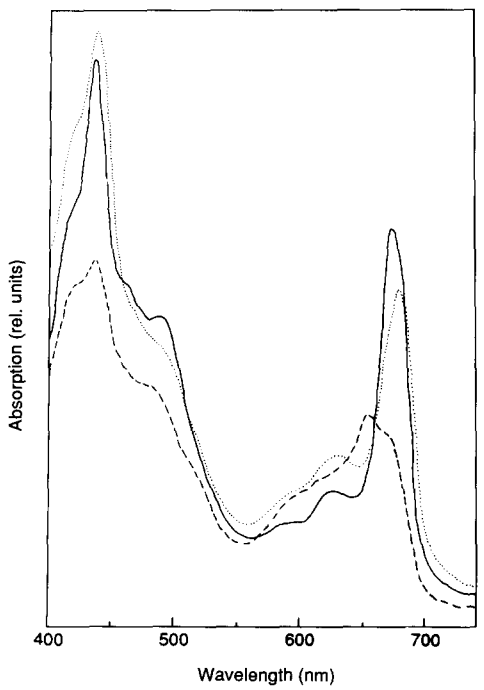

Fig. 1. Absorption spectra of different preparations during isolation of PS-II reaction centers. Membranes (· · · · · ·); SB-12 crude extract (- - - - - -); PS-II particles after purification on Sepharose 6B (———).

Table II gives O_2-evolution activities of oxygen-evolving PS-II particles under different salt conditions. Glycerol was omitted in the assay because it inhibits the O_2-evolution activity up to 30%. The PS-II particles showed a marked response to $MgCl_2$ and $CaCl_2$ when $K_3Fe(CN)_6$ served as electron acceptor. The increase in the rates may be explained by a screening of the negative surface charges of the protein matrix thus facilitating the access for $K_3Fe(CN)_6$ to the acceptor site of the PS-II. Additions of $CaCl_2$ in presence of 10 mM $MgCl_2$ stimulated the activity only when the particles were partially damaged and did not show maximal values (1200–1400 μmol O_2 per mg Chl per h). If the stimulatory effects of Mg^{2+} and Ca^{2+} on $K_3Fe(CN)_6$-media-

TABLE II

EFFECT OF SALTS ON OXYGEN-EVOLUTION ACTIVITY

O_2-evolution activity of crude PS-II particles in 30 mM TEA (pH 7) in the presence of divalent cations at 25 °C. Other conditions as described in Material and Methods.

Electron acceptor	Activity (μmol O_2 per mg Chl per h)
FeCN: control	320
+ $MgCl_2$ (10 mM)	950
+ $CaCl_2$ (10 mM)	1390
+ $Mg/CaCl_2$ (10 mM)	1570
PBQ: control	3720
+ $MgCl_2$ (10 mM)	3700
+ $CaCl_2$ (10 mM)	3800

ted oxygen evolution were predominantly due to an electrostatic screening, almost no Mg^{2+}- or Ca^{2+}-induced increase would be expected in the case of the electrically neutral and lipophilic electron acceptor phenyl-*p*-benzoquinone. This was experimentally confirmed (Table II). In addition phenyl-*p*-benzoquinone also resulted in 2–3-fold higher rates of O_2 evolution, probably due to the higher turnover rate at the Q_B-binding site.

The surface charge of the PS-II particles strongly depends on pH. Therefore, a marked pH-effect is expected for the $K_3Fe(CN)_6$-mediated O_2-evolution, whereas a smaller change should arise for phenyl-*p*-benzoquinone. However, with both acceptors a similar pH-dependence was observed with the maximum rate at around pH = 7.0 (data not shown). This result demonstrates that the pH-dependence of the overall electron transport is dominated by the activity of the enzyme complex itself (as outlined in Ref. 19) rather than by the effects of surface charge on the acceptor at the active site. Surprisingly, the pH optimum measured with PS-II from *M. laminosus* is significantly higher than for other PS-II preparations described. In PS-II preparations from *Synechococcus vulcanus* [20] the optimum was found to be in the acid range (pH = 4.5) whereas similar preparations from *Synechococcus* sp. showed two maxima at pH 6.5 and at pH 4.5 [21]. The basis of this difference remains to be clarified.

Of special interest is the stability of the preparation to detergents. Isolated oxygen evolving PS-II particles were rather stable in octyl-β-D-glucoside and SB-12, but were highly sensitive to LDAO and Triton X-100 which both immediately destroyed the oxygen evolution capacity (see Fig. 2). LDAO or Triton X-100 inhibited electron transport from H_2O to $K_3Fe(CN)_6$ at low concentrations (0.03 and 0.6%, respectively). The half-life time of O_2-evolution activity decreased from 2 min to less than 30 s. Even 25% glycerol or 0.5 M sucrose in the buffer did not stabilize the particles.

If the overall electron-transport rate is limited by the turnover of the exogenous electron acceptor, its concentration should markedly affect the rate of oxygen evolution. Measurements of steady-state electron-transport rates as a function of $K_3Fe(CN)_6$ and phenyl-*p*-benzoquinone concentrations were carried out under optimal conditions for pH and salt concentrations at a temperature of 25 °C. At this temperature the stability of the oxygen-evolving particles was much higher compared to 50 °C, where maximal rates were obtained (vide infra). The highest rates at 25 °C were observed with 0.3 mM phenyl-*p*-benzoquinone. Even with $K_3Fe(CN)_6$ concentrations as high as 10 mM it was not possible to obtain rates comparable to those achieved by phenyl-*p*-benzoquinone. This finding differs from observations in Ref. 21 where 10 mM $K_3Fe(CN)_6$ gave activities similar to phenyl-*p*-benzoquinone.

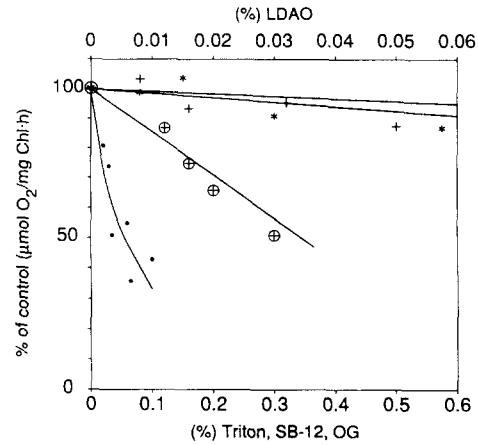

Fig. 2. Effect of the concentration of different detergents on O_2-evolution activity of crude PS-II particles. The samples containing 10 μg chlorophyll were incubated in the oxygen-electrode chamber in a final volume of 3 ml containing various amounts of detergents. SB-12 used for solubilization had a final concentration of less than 0.01%. After an incubation of 1 min $K_3Fe(CN)_6$ was added, the O_2-evolution activity was measured in the light for 2 min. •, Triton X-100, +, SB-12, *, OG, ⊕, LDAO.

The electron transport of the oxygen-evolving particles was strongly dependent on temperature. The highest rates of O_2 evolution were observed at 50 °C with $K_3Fe(CN)_6$ and at 45 °C with phenyl-*p*-benzoquinone as electron acceptor, with an 2–3-fold increase of the rates between 25 and 50 °C (Fig. 3). At higher temperatures the activities sharply declined with time even in the presence of stabilizing 0.5–1 M sucrose or 25% glycerol. Maximal rates were between 45 and 50 °C; however, there the electron-transport rates remained constant for only about 30 s. This temperature of

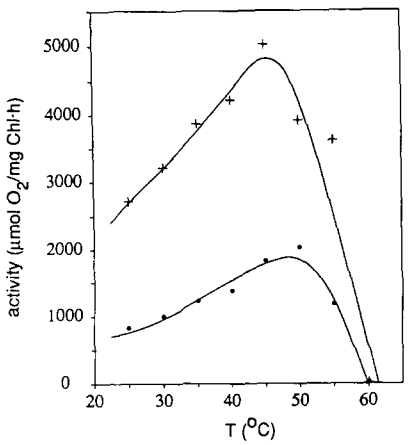

Fig. 3. Effect of temperature on oxygen-evolution activity of crude PS-II particles. The measurements were done as described in Material and Methods. Reaction medium: buffer 2 plus 0.5 M sucrose. The rate was calculated from the change in oxygen concentration during the first 30 s after onset of illumination.

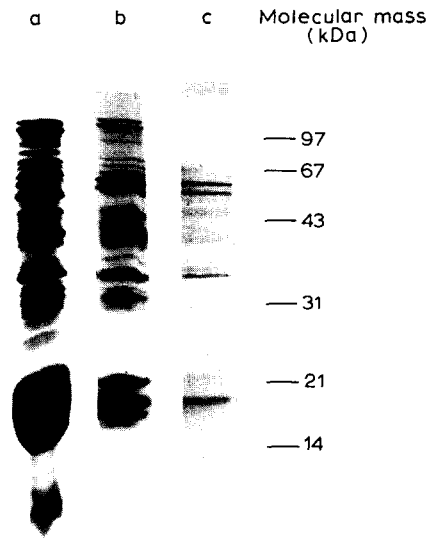

Fig. 4. SDS-polyacrylamide gel electrophoresis profiles of *M. laminosus* PS-II particles. (a) Crude extract; (b) oxygen-evolving particles after gelfiltration; (c) non-oxygen-evolving particles after anion-exchange chromatography. Samples were prepared and electrophoresis carried out as described in Material and Methods. The gel was stained with Coomassie blue. Marks on the right side indicate positions of rabbit muscle phosphorylase *b* (97 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), bovine carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21 kDa) and hen egg white lysozyme (14 kDa), respectively.

maximum electron-transport rate correlates with the growth temperature of the cells of 50 °C. These results indicate that the oxygen-evolving complex in this alga is stable at these high temperatures as in *Synechococcus* sp. [21] and *Synechococcus vulcanus* [20]. Fig. 4 shows typical profiles of SDS-polyacrylamide gel electrophoresis, of the crude extract (a), the purified oxygen-evolving particles (b) and of the purified non-oxygen-evolving PS-II complex (c). The oxygen-evolving particles consist of nine major proteins: two large polypeptides of apparent molecular masses of about 110 and 105 kDa, two of 60 and 55 kDa, two of 33 and 35 kDa, and three small polypetides of 22, 18 and 17 kDa.

The purification step with anion exchange chromatography leads to a preparation which had lost the oxygen-evolving capacity. This PS-II complex shows four major polypeptides of the following molecular masses (in kDa): 60, 55, 35 and 18. In both preparations the chlorophyll migrated as free chlorophyll.

Laser-flash-induced absorption changes

A true characterization of the PS-II particles requires information about the antenna size (e.g., the ratio of Chl/P-680), about the coupling between the water-splitting enzyme system Y and the primary donor P-680 (characterized by the reduction kinetics of $P\text{-}680^+$) and about the integrity of the acceptor side of PS II. This goal can only be achieved by time-resolved flash spectroscopy. Therefore we measured laser flash (FWHM, approx. 7 ns) -induced absorption changes at 830 nm and at 325 nm. The former are due to the transient photo-oxidation of the primary donor of the PS-II reaction center P-680, the latter predominantly to the turnover of the plastoquinones at the PS-II acceptor side with minor contributions from the donor side reactions. Typical absorption changes at 830 nm observed under repetitive laser flash excitation of oxygen-evolving PS-II fragments are depicted in Fig. 5b. The relaxation kinetics of 830 nm absorption changes which reflect the reduction of $P\text{-}680^+$ exhibit a multiphasic pattern in the nano- and microsecond

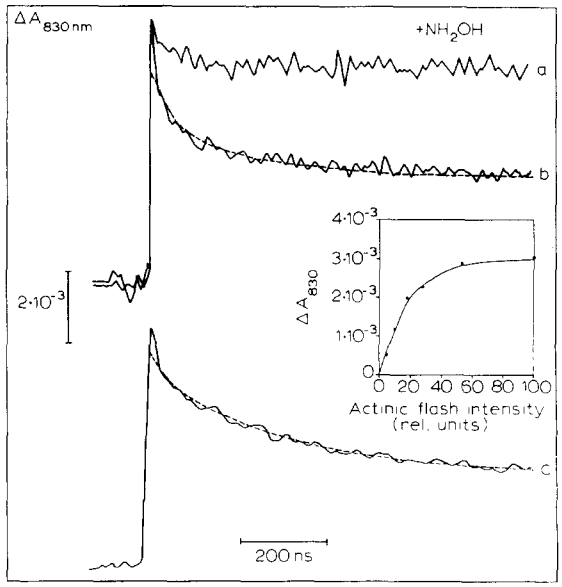

Fig. 5. Time-course of absorption changes at 830 nm induced by repetitive laser flashes: (a) oxygen-evolving particles treated with 3 mM NH_2OH; (b) oxygen-evolving particles; (c) non-oxygen-evolving particles. Conditions: $8 \cdot 10^{-5}$ M Chl; buffer 1 (pH 7); 1 mM $K_3Fe(CN)_6$ as electron acceptor; optical pathlength, 4 cm; nonsaturating laser flash, 530 nm; FWHM, 7 ns, repetition rate 2 Hz; average 64 sweeps. The broken line was calculated using two exponential phases for the nanosecond time range. Inset: Initial amplitude of 830 nm absorption changes as a function for the excitation energy in oxygen-evolving particles. In order to achieve saturation these measurements were carried out in a cuvette with 1 cm pathlength at a chlorophyll concentration of 40 μM. Other conditions as above.

range [13,22]. The NH_2OH-induced retardation of the 830 nm relaxation kinetics (Fig. 5a) and the greatly diminished initial signal amplitude in the presence of DCMU (data not shown) indicate that Chl triplets do not significantly contribute to the 830 nm absorption changes. In our preparation the relative contribution of the nano- and microsecond components to the overall P-680$^+$ reduction is of the same magnitude (0.53 : 0.47). A more refined analysis reveals, however, that the nanosecond kinetics contain at least two phases of approx. 30 ns and 200 ns half-life time with amplitudes normalized to the total extent of 0.28 and 0.25, respectively. Furthermore, a small rapid transient with a half decay time of less than 8 ns is observed in Fig. 5b. Although this component is partly sensitive to NH_2OH incubation (Fig. 5a) it has not been taken into account because the time resolution of the instrument was limited to about 8 ns. Preliminary investigations with a time resolution of about 500 ps (35 ps laser pulse excitation; electrical bandwidth, 700 MHz) indicate that the half-life time of this component is about 5 ns (data not shown).

The microsecond kinetics could be fitted by a triphasic exponential decay with the following half-life times and normalized amplitudes (in brackets): 4 μs (0.20), 35 μs (0.15) and 250 μs (0.13). These results qualitatively correspond to previous findings in *Synechococcus* particles [23] and spinach thylakoids [24]. After inhibition of the oxygen-evolving activity by NH_2OH treatment the nanosecond kinetics almost completely disappear parallel to an increase of reactions in the microsecond time scale. This raises the question whether the extent of the nanosecond kinetics directly correlates with the number of functionally competent water oxidizing enzyme systems. The 830 nm absorption changes observed with particles which have lost their oxygen-evolving capacity by purification with an anion-exchange column are depicted in Fig. 5c. A comparison with the data of Fig. 5b readily shows that the total extent of the nanosecond kinetics is minimally affected by the purification procedure, in spite of the oxygen-evolving capacity being almost completely lost. The relaxation kinetics are slightly slower in this preparation due to an increase of the normalized extent of the 200 ns kinetics (0.40) occurring at the expense of the 30 ns kinetics (0.15).

In order to check the percentage of System II functionally competent in water cleavage, the amplitude of the 830 nm absorption change and of the average oxygen yield per flash were measured under saturating excitation conditions. In the inset of Fig. 5 the initial amplitude of the 830 nm absorption change is depicted as a function of the actinic flash intensity. Using a difference extinction coefficient $\Delta\varepsilon_{830}$ (P-680$^+$/P-680) of 6000 $M^{-1} \cdot cm^{-1}$ [30] the saturation level was calculated to correspond to a ratio of 75 chlorophylls per P-680. Likewise, the average oxygen yield per flash indicates a ratio of one intact water-oxidizing enzyme system per 80–100 chlorophylls. An examination of these data shows that 75–95% of the

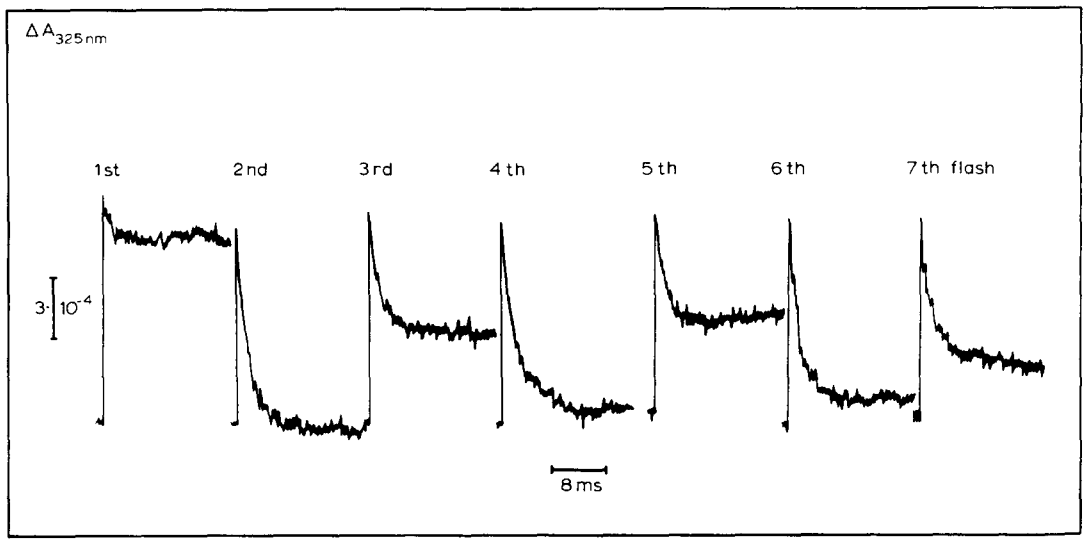

Fig. 6. Time-course of absorption changes at 325 nm as a function of flash number in 2 min dark-adapted oxygen-evolving particles. Conditions: $1 \cdot 10^{-5}$ M Chl; buffer 1 (pH 7); 1 mM $K_3Fe(CN)_6$; optical pathlength 1 cm; average, 32 sweeps; darktime between flashes, 500 ms.

System II remains functionally intact during the isolation procedure described in this study. However, after the anion-exchange chromatography the oxygen-evolution capacity largely disappears.

Fig. 6 illustrates typical traces of 325 nm absorption changes induced by a flash train in 2 min dark-adapted oxygen-evolving samples. These absorption changes are dominated by the turnover of the primary plastoquinone acceptor Q_A (70–80%). However, reactions at the donor side of PS-II may interfere. Interpretation of the data is strongly dependent on the time resolution of the measurements (for details see Refs. 25 and 26). The observed characteristic binary oscillation pattern due to the plastoquinol formation at the acceptor side of PS-II indicates an unaffected electron transfer from Q_A to Q_B in these samples. Interestingly, in the non-oxygen-evolving preparations the binary oscillation largely disappears (Fig. 7).

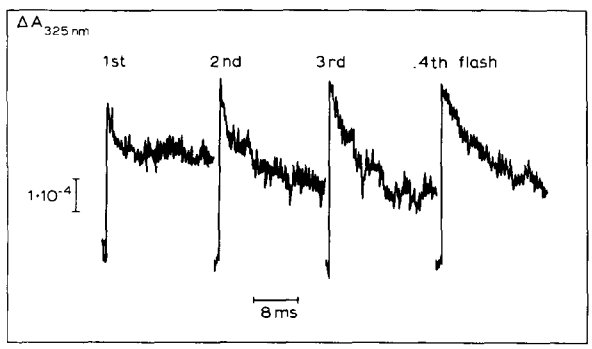

Fig. 7. Time-course of absorption changes at 325 nm as a function of flash number in 2 min dark adapted non-oxygen-evolving particles. Other conditions as in Fig. 6.

Discussion

The present study shows that PS-II particles can be isolated by SB-12 treatment of thylakoid membranes of *M. laminosus* which retain a high oxygen-evolving capacity. A successful isolation depends on the physiological state of the cells as well as on the presence of glycerol and bivalent cations. These findings indicate that the state of the membrane and the proper balance of hydrophobic and hydrophilic interactions between membrane compounds and solubilizing medium is of crucial importance for the isolation of active material. In this respect it is interesting to note that other detergents like LDAO and Triton X-100 which are very efficient for the isolation of PS-II fragments from other cyanobacteria [3] and from chloroplasts [27] rapidly destroy the oxygen-evolv-

ing capacity of PS-II preparations of *M. laminosus*. Accordingly, special structural requirements have to be satisfied by the detergents in order to permit solubilization and simultaneously to retain the functional integrity. The underlying mechanisms for these interacting effects remain to be clarified.

The PS-II fragments solubilized from *M. laminosus* membranes with sulfobetaine not only retain the oxygen-evolving capacity but also the integrity of the Q_B-binding site is preserved to a large extent. This conclusion is based on two findings: (a) phenyl-*p*-benzoquinone acts as a very efficient electron acceptor in a highly DCMU-sensitive reaction; (b) the laser-flash-induced 325 nm absorption changes exhibit a characteristic binary oscillation. It has to be emphasized that the oscillation pattern of the PS-II particles of *M. laminosus* markedly differs from that observed under similar conditions in spinach PS-II fragments [33]. The reason for this difference requires further detailed kinetic analysis which is beyond the scope of the present study. The binary oscillation disappears in anion-exchange column-purified samples which have lost their oxygen-evolution capacity. This indicates that the purification step does not only affect the catalytic site of the water oxidation but also the Q_B binding site. The markedly slower rate achieved with $K_3Fe(CN)_6$ and the effect of bivalent cations (Table II) suggest that the microenvironment of the site of interaction with the exogenous electron acceptor is negatively charged.

A very interesting result of the present study is the finding that the oxygen-evolving capacity is almost completely eliminated in anion-exchange purified samples with only marginal effects on the relative extent of the nanosecond kinetics of P-680$^+$-reduction (Fig. 5c). A similar observation has been reported recently with PS-II preparations from spinach chloroplasts [28]. PS-II fragments from spinach chloroplasts loose oxygen evolution almost completely by treatment with lauroylcholinchloride (LCC) or with DIDS while ΔA_{ns}^{rel}(P-680$^+$) is little affected even under repetitive flash excitation. This phenomenon implies mechanistic questions about the type of modification of the water-oxidizing enzyme Y. The P-680$^+$ reduction kinetics have been shown to be markedly changed when the enzyme system Y is seriously damaged [25,28–33]. Under repetitive flash excitation P-680$^+$ is reduced in the dark in NH_2OH or Tris-treated samples either from Q_A^- [29,30] or from Z [25,31–33] depending on the degree of Z regeneration between the flashes. Therefore, the effect described here and in Ref. 28 is likely to be caused by a different type of modification of the Y complex or of the functional coupling between Y and P-680. It seems reasonable to assume that structural changes induced by the treatments mentioned above are much less drastic than in NH_2OH- or Tris-treated samples. Alternatively, one could assume that the loss of oxygen evolution in the purified samples is solely due to an inhibition of the electron transfer on the acceptor side of PS II accompanied by a reoxidation of Q_A^- by backreaction with the S_2 and/or S_3 state of the water-oxidizing enzyme system between the flashes. However, under repetitive flash excitation this would lead to an increased population of the S_1 state, and hence to an acceleration of the P-680$^+$ reduction to a 25 ns kinetics, since the P-680$^+$ reduction kinetics depends on the redox state of the water-oxidizing enzyme system [34]. This is not observed in the purified samples (see Fig. 5c). Albeit the mechanistic details of the inhibition of oxygen evolution remain unsolved, the present study reveals that the relative contribution of the nanosecond kinetics to the overall P-680$^+$-reduction cannot be used as an unambiguous indicator of the functional integrity of the water-oxidizing enzyme Y, a conclusion which is in agreement with recent experiments of Renger and coworkers [14].

The SDS-polyacrylamide gel electrophoresis analysis shows for the oxygen-evolving particles and for the non-oxygen-evolving particles 9 and 4 major protein bands, respectively. The two polypeptides of molecular mass, 110 kDa and 105 kDa which are found in the oxygen-evolving particles, were also found in Ref. 4 and are suggested to be a trimeric $(\alpha\beta)_3$ aggregate of allophycocyanin. This agrees with the observation that allophycocyanin is still found in the preparation after gelfiltration. The 17 kDa and the 22 kDa polypeptides are the subunits of the phycobiliproteins. After anion exchange chromatography the preparation was free of allophycocyanin and the two large subunits of 110 and 105 kDa and the small

polypeptides of 17 and 22 kDa had been removed. There is great similarity between the proteins of *M. laminosus* and *Phormidium laminosum* [35] and *Synechococcus cedrorum* [2]. A striking difference in the preparation of *M. laminosus* is a lack of the 48 and 43 kDa polypeptides, which are typical for many purified PS-II preparation reported from Cyanobacteria [36,37]. Nanba et al. [38] isolated a PS-II complex that showed charge separation even when the 47 kDa and the 43 kDa polypeptides were missing. A similar behaviour was observed in our preparations. The site of the primary charge separation in our preparations has not been determined yet.

The present study demonstrates that the PS-II particles obtained from *M. laminosus* as described provide an interesting source of material for further studies of the reaction mechanisms of PS II.

Acknowledgements

This work was supported by the Swiss National Foundation, grant No. 3.243-0.85, by the Deutsche Forschungsgemeinschaft (Sfb 312) and by the European Molecular Biology Organization (travel support).

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