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Isolation and characterization of oxygen-evolving Photosystem II membranes from the cyanobacterium *Synechocystis* 6803

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A simple and rapid procedure has been designed for isolation of a highly active Photosystem II (PS II) subfraction from the cyanobacterium *Synechocystis 6803*. The thylakoid membranes used as starting material were isolated from cells treated with red light to increase the relative PS II content. The cell walls were broken in a glass-bead cell disruptor. The isolated thylakoids were pretreated with 0.01% dodecyl β-maltoside, followed by solubilization in 2.5% Triton X-100 to selectively extract photosystem I. The membrane residue, still containing PS II, was collected by a rapid differential centrifugation. The isolation PS II membranes possess rates of oxygen evolution as high as 2800–3200 μmol O_2/mg Chl h, and a low proportion of contaminating photosystem I. The yield of PS II is 30–35% on a PS II activity basis. Typically, PS II membranes corresponding to 2 mg of chlorophyll could be obtained within 6 h of preparative work, including the cell breakage. The purity and high intactness of the material allowed EPR analysis of various PS II parameters, including signal II_{slow} and the S_2 state multiline signal. Considering the simplicity of the subfractionation procedure, the high yield and functional intactness, this preparation should have the potential of playing the same important experimental role for studying PS II in *Synechocystis 6803* as the so called BBY-preparation has played for studies on PS II from plants. In particular, the preparation should be essential for studies on structure-function relations after site-directed mutagenesis of various PS II subunits in *Synechocystis 6803* cells.

Introduction

A number of recent advances in photosynthesis research have contributed to the understanding of the molecular structure and function of Photosystem II (PS II) of higher plants and cyanobacteria. The crystallographic determination of the three-dimensional structure of photosynthetic purple bacteria reaction centres [1], together with isolation of a homologous reaction centre complex from higher plants [2,3] have enabled detailed predictions of PS II reaction centre structure [4–7]. For experimental studies on the structural and functional aspects of PS II, the use of molecular biology methods such as site-directed mutagenesis and gene deletion has increased [8,9].

In particular, the cyanobacterium *Synechocystis* 6803 has proven to be very suitable for studies of PS II, since this strain is a facultative photoheterotroph [10] which is naturally competent for transformation [11]. This means that it possesses a mechanism for uptake of exogenous DNA and incorporation into the chromosome by homologous cross-over. *Synechocystis* 6803 has already been used for construction of a number of PS II mutants [12–18]. However, this approach is limited by the lack of adequate biochemical procedures for preparation of active, oxygen-evolving PS II membranes from *Synechocystis* 6803. One important criterion for these preparations must be, besides possessing a functional photosynthetic apparatus, that they are sufficiently pure to allow finely tuned spectroscopic analysis, in particular EPR, of various PS II components.

There are two major problems with obtaining such a preparation; the difficulty of maintaining functional water-oxidation throughout the preparation and the removal of contaminating PS I, which tends to mask

Abbreviations: Chl, chlorophyll; DMSO, dimethyl sulphoxide; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; Mes, 4-morpholineethanesulphonic acid; P_{700}, primary electron donor of PS I; PAGE, polyacrylamide gel electrophoresis; PpBQ, phenyl-*p*-benzoquinone; PS, photosystem; SDS, sodium dodecyl sulphate; Tes, *N*-tris(hydroxymethyl)-methyl-2-aminoethanesulphonic acid; Tyr_D, tyrosine 160 of the D2 polypeptide; Tyr_Z, tyrosine 161 of the D1 polypeptide.

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EPR signals originating from PS II. The latter difficulty is partly due to the fact that the thylakoid membrane of *Synechocystis* 6803 may contain up to 5 times as much PS I as PS II [19].

In this work we have selectively extracted PS I from the thylakoid membranes of *Synechocystis* 6803 by a mild solubilization procedure and isolated highly active PS-II-enriched membranes. The procedure is simple and rapid, has a high yield and results in a PS II preparation which has low PS I content and is suitable for EPR spectroscopy.

Materials and Methods

Triton X-100 (purified for membrane research) was obtained from Boehringer–Mannheim and dodecyl β-D-maltoside from Calbiochem.

Cell cultivation

The *Synechocystis* 6803 cells were grown at 33°C in BG-11 medium with 5 mM Tes (pH 8.0) in 10 litre flasks under a 5% CO_2/air atmosphere and subjected to white light (100 μE cm^{-2} min^{-1}) [20]. When the culture reached late log phase the light source was fitted with red filters (cut off 650 nm). The cells were harvested after 12 h of red-light treatment.

Thylakoid preparation

Thylakoid membranes were prepared (Fig. 1) by a modified version of the method described by Nilsson et al. [21], in which the cells are incubated in a medium with a high sucrose concentration [22], and the cell-wall broken by homogenization in a glass-bead cell disruptor [19]. The cells were harvested in late log phase and washed once in medium A (50 mM Hepes (pH 6.5), 10 mM $MgCl_2$, 30 mM $CaCl_2$, 1 M sucrose, 25% (v/v) glycerol), and resuspended to a concentration of 0.5 mg Chl/ml. After 1 h incubation on ice, 2 g of glass beads (Braun–Melsungen, 0.17–0.18 mm) were added for every ml of the suspension and cells were subjected to 15 disruptions of 20 s spaced with 4 min cooling intervals in a 'Bead-Beater' homogenisator (Biospec Products) operated at 2°C. Unbroken cells and cell debris were pelleted by centrifugation at $6000 \times g$ for 20 min. The pellet was resuspended and centrifuged again to reclaim thylakoid membranes cosedimenting with the intact cells. The two supernatants were pooled and centrifuged at $100000 \times g$ for 45 min. The pellet was resuspended in medium B (50 mM Hepes (pH 6.5), 10 mM $MgCl_2$, 30 mM $CaCl_2$, 0.2 M sucrose, 25% (v/v) glycerol), containing 5% (v/v) DMSO. The isolated thylakoid membranes were stored in liquid nitrogen for further use.

Preparation of PS II membranes

The thylakoid preparation was suspended in medium B to a concentration of 3.0 mg Chl/ml. Dodecyl β-maltoside (from a 0.5% stock solution) was added to a final concentration of 0.01% (w/v) and the suspension was gently agitated for 1 min. An equal volume of medium B containing 5% (w/v) Triton X-100 was added to the thylakoid suspension and the mixture was gently stirred for 30 s. Twice the initial volume of medium B containing 10 mM $LiClO_4$ was added and the suspension was centrifuged at $20000 \times g$ for 15 min. The pellet was discarded. The supernatant was collected and centrifuged at $100000 \times g$ for 45 min (Fig. 1). All preparation steps were performed at 4°C and dim light. The pelleted material was finally resuspended in medium B, frozen in liquid nitrogen and stored at −80°C.

By increasing the Triton X-100 concentration in medium B to 5.2%, giving a final concentration of 2.6% during the solubilization, the purity of the PS II preparation could be increased. However, in these high-purity preparations the yield decreased.

Characterization of the preparation

During the initial optimization of the detergent solubilization conditions, the efficiency of the different solubilization procedures were monitored by sucrose gradient centrifugation, using gradient systems from 0.2–2 M sucrose in 50 mM Hepes (pH 6.5), 10 mM $MgCl_2$, 30 mM $CaCl_2$, 0.2 M sucrose, 25% (v/v) glycerol with addition of appropriate detergents.

PS II oxygen-evolving activity was assayed at 22°C using a Clark-type electrode. 77 K fluorescence emission spectra were recorded using a Perkin-Elmer LS 50 fluorescence spectrophotometer. Samples containing 20 μg Chl/ml were excited at 433 ± 5 nm.

P_{700} content was estimated by measuring light-induced absorbance changes at 700 nm. Absorbance difference spectra between ascorbate-reduced and light-oxidized samples were recorded using a Shimadzu UV-3000 spectrophotometer. An absorption coefficient of 64 mM^{-1} cm^{-1} was used to calculate the P_{700} content [23].

SDS-PAGE was performed in the buffer system of Laemmli [24] at 4°C with a 12–22.5% polyacrylamide gradient in the presence of 4 M urea [25].

EPR spectroscopy

X-band low-temperature and room-temperature EPR spectra were recorded at 9.239 and 9.77 GHz microwave frequency, respectively, with a Bruker ESP-300 spectrometer equipped with an Oxford Instruments liquid He cryostat. The data were processed using the ESP-300 program. The samples were illuminated, when required, at 200 K for 10 min with a 1000 W projector, using an unsilvered Dewar containing solid CO_2/ethanol for cooling. EPR Signal II_{slow}, from $Tyr_D^{\cdot}$, was measured in the dark whereas Signal II_{fast}, from $Tyr_Z^{\cdot}$, and Signal I from $P_{700}^{+\cdot}$ in PS I were

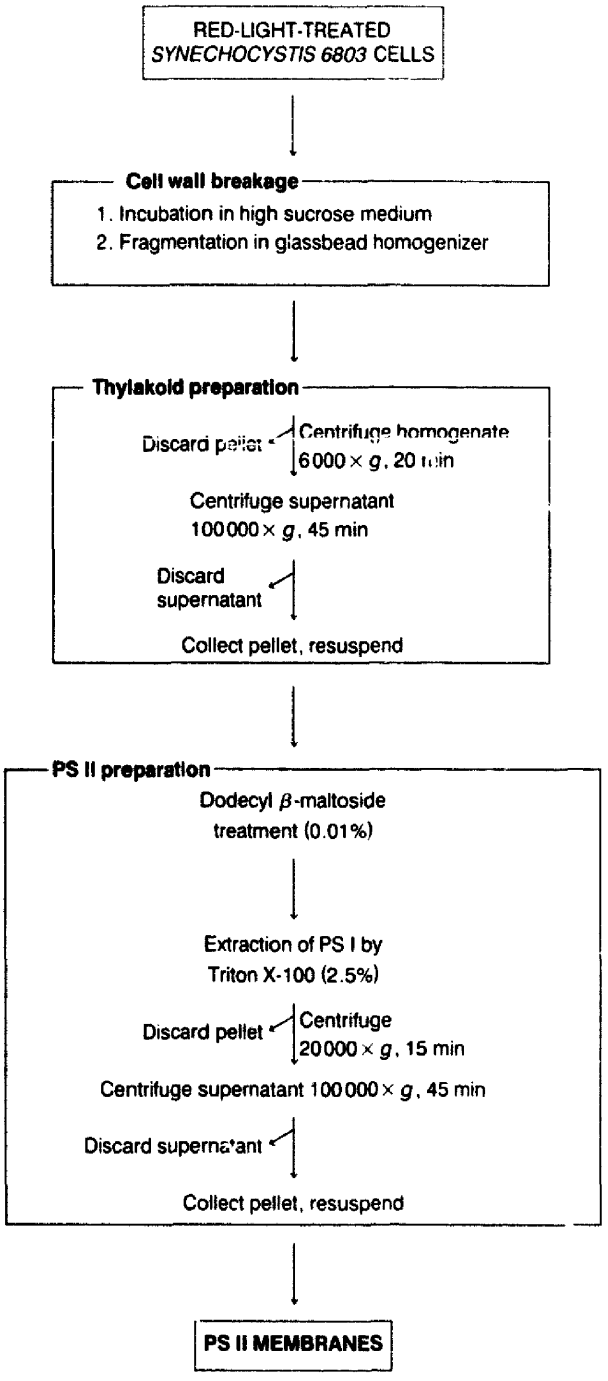

Fig. 1. Preparation scheme for thylakoids and oxygen-evolving PS II membranes from *Synechocystis* 6803.

recorded at room temperature under continuous illumination using heat-filtered saturating white light.

Results

Cell cultivation

The high relative PS I content in *Synechocystis* 6803 is a major problem when purifying PS II particles. To increase the proportion of PS II in the thylakoids we subjected the cells to red light the last 12 h prior to harvesting. Red light is predominantly absorbed by PS I and consequently the relative PS II content is increased to compensate for the unbalanced excitation. This treatment of the cells increased the oxygen evolution relative to chlorophyll concentration in the isolated thylakoid preparation with approx. 70%, from about 300 μmol O_2/mg Chl per h to about 500 μmol O_2/mg Chl per h. Prolonged periods of red light treatment did not further increase the relative PS II content as judged by measurements of oxygen evolution.

Thylakoid preparation

The thylakoid preparation is based on a procedure described earlier in Ref. 21, using a glass-bead homogenizer for disruption of the cyanobacterial cell wall [19]. The following improvements were adopted to improve the quality of the isolated thylakoids. 1 M sucrose was included in the preparation medium, since incubation in a medium with high sucrose concentration prior to fragmentation allows for prolonged disruption intervals and increases the yield without loss of PS II activity [22]. Increasing the length of cooling intervals between the disruptions from 1.5 min to 4 min allowed the number of disruptions to be increased from 5 to 15 without loss of PS II activity. Moreover, the homogenized cells were centrifuged for a longer time to ensure that no intact cells or cell debris contaminated the thylakoid membrane preparation. The oxygen-evolving activity of this preparation was about 500–600 μmol O_2/mg Chl per h and the yield on a chlorophyll basis was as high as 80%.

Preparation of oxygen-evolving PS-II-enriched membranes

The PS II preparation was isolated from the cyanobacterial thylakoid membranes by a combination of dodecyl β-maltoside and Triton X-100 detergent treatment and a simple differential centrifugation procedure (Fig. 1). A typical preparation has a yield of approx. 30% based on PS II oxygen-evolving activity and possesses rates as high as 2800–3200 μmol O_2/mg Chl per h (Table I). The functional intactness, in combination with a relatively low PS I contamination, makes the material suitable for EPR analysis (see below).

Initially a number of different detergent treatments were tested with the aim of finding conditions for selective extraction of PS I from the membrane without loss of oxygen-evolving capacity. Triton X-100, dodecyl β-maltoside, SB 12 and octyl β-D-glucoside were all tried at different concentrations and combinations. Their ability to extract PS I was monitored by sucrose

gradient centrifugation, and the intactness of PS II was assayed by measurement of oxygen evolving rates. Triton X-100 solubilization resulted in an appreciable extraction of PS I from the membrane, but also in a severe loss of oxygen evolving activity. We could not obtain a selective extraction of PS I in appreciable amounts using the other detergents. It was found that the best solubilization conditions were obtained by 2.5% Triton X-100 in combination with a pretreatment with 0.01% dodecyl β-maltoside (Fig. 1). Due to the natural variability in the thylakoid material the solubilization procedure has to be carefully optimized for different batches by a small-scale test preparation, unless care is taken to grow the *Synechocystis* cells and prepare thylakoids under highly reproducible conditions.

Initially sucrose gradient centrifugation for a prolonged period (up to 16 h) was used to isolate the PS II membranes following solubilization. This resulted in a pronounced decrease of oxygen-evolving activity. In order to minimize the time required for centrifugation, a simple and rapid fractionated centrifugation procedure was designed (Fig. 1). It was found that in the presence of 200 mM sucrose at $100000 \times g$ for 45 min, the PS II enriched membranes sedimented, whereas most of the PS I was found in the supernatant.

The entire preparation could be completed within 6 h, including the time required for cell breakage and isolation of thylakoids. Typically, a preparation starting from cells containing 50 mg chlorophyll yielded PS II membranes containing 2 mg chlorophyll. Starting from thylakoids, the preparation can be completed in 90 min.

Characterization of isolated PS II membranes

Oxygen evolution rates of approx. 2800 μmol O_2/mg Chl per h were frequently obtained (Table I). Rates as high as 3200 μmol O_2/mg Chl per h could be obtained by increasing the final Triton X-100 concentration to 2.6% (Table I).

The yield, based on PS II oxygen-evolving activity, varies from about 30–35% down to 1–2% depending on the desired purity. A typical high-yield preparation usually has a PS I : PS II ratio of about 1 : 3–4, which is sufficiently pure to allow for EPR measurements of PS II signals.

P_{700} was measured in the PS II membranes to estimate their PS I contamination (Table 1). The Chl/P_{700} ratio was increased to 890 in the PS II isolated membranes from approx. 150 in the initial red-light treated thylakoids. These values demonstrate a pronounced degree of purification of PS II, although it is difficult to translate them directly to purification efficiency, since different antenna sizes for PS I in *Synechocystis* 6803 have been reported and PS I may lose chlorophyll during the extraction procedure [26,27].

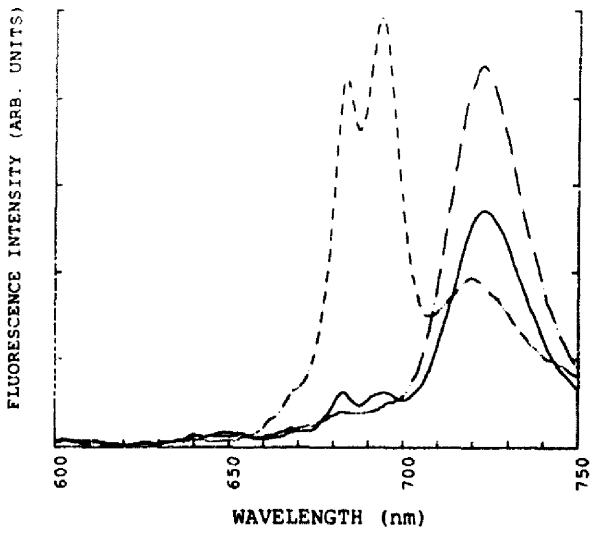

Fig. 2. 77 K fluorescence emission spectra obtained from PS II membranes (· - - - ·) and thylakoids prepared from white-light-grown (— · —) and red-light-treated (———) *Synechocystis* 6803 cells. The excitation wavelength was 433 ± 5 nm and the samples contained 20 mg Chl/ml.

Another method to estimate the purity of the isolated PS II membranes is by 77 K fluorescence emission measurements, using an excitation wavelength of 433 nm (Fig. 2). The emission maxima at 685 nm and 695 nm are emitted by PS II chlorophyll *a* species, whereas the 720 nm maximum is emitted by chlorophyll *a* associated with PS I [28]. Spectra obtained from thylakoids prepared from cells not subjected to red light prior to harvesting show only weak PS II emission (Fig. 2), whereas in thylakoids from red-light treated cells the PS II emission is enhanced. The spectrum obtained from PS II membranes is clearly dominated by the fluorescence maxima at 685–695 nm, whereas the PS I emission at 720 nm remains only as a minor shoulder (Fig. 2).

SDS-PAGE analysis of the PS II preparation resolves approx. 25 polypeptides and shows a considerably simpler polypeptide pattern compared to the starting thylakoid material (Fig. 3). This is due principally to the removal of phycobilisome proteins and PS I subunits. Only a weak, diffuse band could be detected in the 60–70 kDa region, which may represent *psa*A and *psa*B gene products from residual contaminating PS I [29]. This rather complex polypeptide pattern, together with the fact that PS II material is easily pelleted, suggests that the PS II preparation consists of membrane sheets rather than individual protein complexes. This is further supported by electron microscopy analysis, which reveals the presence of membrane sheets in the preparation (not shown).

Fig. 3. Polypeptide analysis of the PS-II-enriched membranes (lane A) and thylakoid membranes (B) from *Synechocystis* 6803 by SDS-PAGE in the prescence of 4 M urea.

EPR spectroscopy

Signal II_{slow} is an useful EPR signal for the donor side of PS II that originates from a dark-stable oxidized tyrosine residue, Tyr_{D}^{+}, on the D2 protein (D2Tyr160) [30,31]. Both in the unfractionated thylakoids and the isolated PS II membranes the spectrum of Signal II_{slow} has the same g-value ($g = 2.0046$) and linewidth (18 G) as for PS II in higher plants. Similar to the situation in other cyanobacteria, the spectrum is less well-resolved than in higher plants.

As an additional probe for the purification of PS II from PS I we measured the EPR spectrum in the radical region both in the dark and in the light. Oxidized tyrosine/chlorophyll was estimated by comparing Signal II_{slow} in the dark in thylakoids and PS II membranes obtained from *Synechocystis* 6803 with that of PS II membranes from spinach thylakoids. The PS II membranes from spinach contains 220–250 chlorophylls per PS II reaction centre [32]. In the starting thylakoid membranes the Signal II_{slow} in the dark amounts to one oxidized tyrosine per approx. 650–750 chlorophylls, whereas in the PS II particles it amounts to one oxidized tyrosine per 45–55 chlorophylls.

Continuous illumination at room-temperature results in the oxidation of P_{700}. This has a featureless radical signal at $g = 2.0026$ (linewidth 7–8 G) which dominates the spectrum recorded under illumination in the thylakoid membranes (Fig. 4A, top spectrum). Double integration of the spectra recorded in the dark and during illumination reveals that the PS II to PS I ratio in the thylakoids was about 0.2. Thus, the EPR signal from P_{700}^{+} totally dominates the EPR spectrum under illumination, which is one of the main reasons why unfractionated thylakoids from *Synechocystis* 6803 are quite unsuitable for EPR studies on the donor side of PS II. In the purified PS II membranes, however, the illumination induced only a small signal from P_{700}^{+} on top of Signal II_{slow} (Fig. 4B). The ratio of PS II to PS I in the isolated PS II membranes depends on the detergent treatment. In the preparation used in Fig. 4B

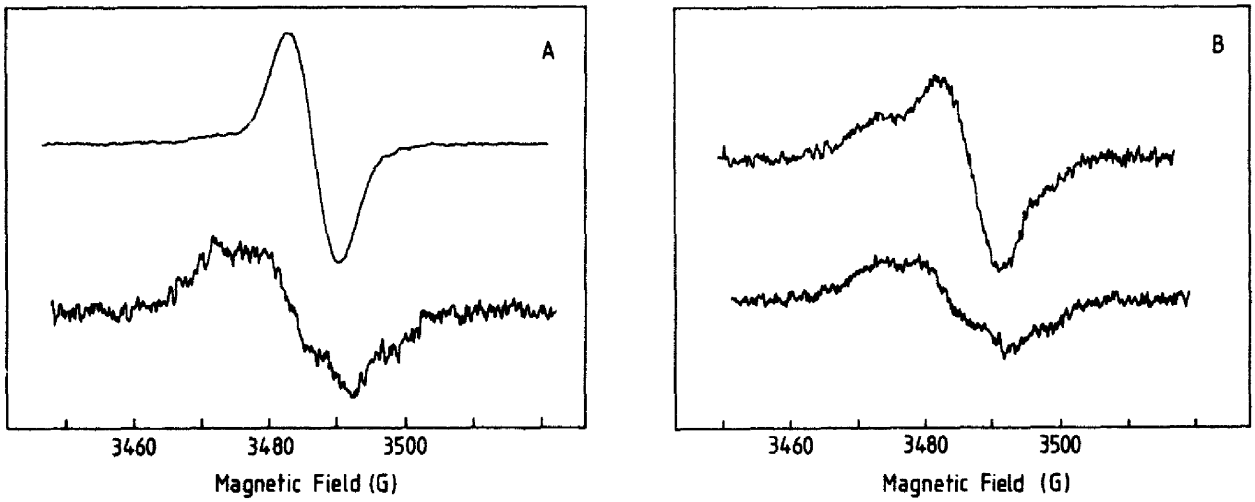

Fig. 4. EPR spectra of the radical region recorded; (A) Thylakoid membranes; (B) PS II membranes. The EPR spectra were recorded in the dark (lower spectrum), or under continuous illumination (upper spectrum) with heat-filtered white light from a 1000 W projector. Note the 8-times larger magnification in the dark spectrum as compared to the illuminated spectrum in (A). The chlorophyll concentration was (A) 2.2 mg Chl/ml, and (B) 0.7 mg/ml. EPR conditions: $T = 293$ K; microwave frequency, 9.77 GHz; microwave power, 3 mW; modulation amplitude, 3.2 G.

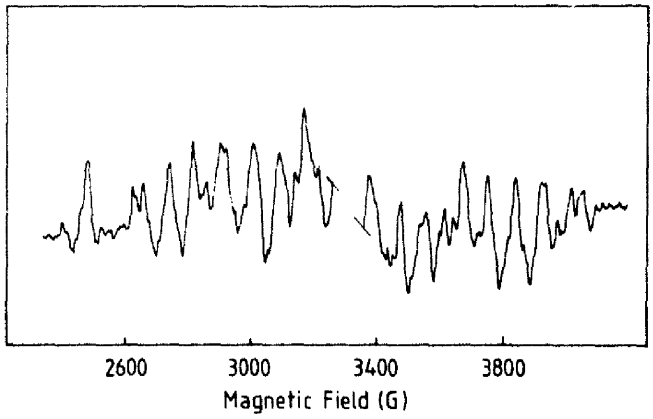

Fig. 5. The S2-state multiline EPR signal recorded in a purified PS II preparation from *Synechocystis* 6803. The spectrum was recorded after illumination at 200 K. The spectrum shown is the illumination-dark difference spectrum. EPR conditions: T = 10 K; microwave frequency, 9.223 GHz; microwave power, 20 mW; modulation amplitude, 20 G.

the PS II to PS I ratio is about 3:1 compared to 1:5 for unfractionated thylakoids.

In the illuminated spectrum obtained from these membranes (Fig. 4B), the low-field shoulder of Signal II_{slow} (at 3470 G) is free from the PS I radical signal. This shows that the preparation at this purity will be useful for kinetic EPR studies of Tyr_D· and Tyr_Z·. The purity can easily be improved to almost negligible PS I levels, resulting in an almost total loss of the EPR Signal I, but in this case the yield of membranes is reduced.

A useful indicator for the intactness of the donor side in PS II is the presence or absence of Signal II_{fast} during illumination. Tyr_Z of the D1 protein in its oxidized form gives rise to a similar EPR spectrum as Signal II_{slow} [33]. However, it decays considerably faster and in oxygen-evolving PS II centres the spectrum from Tyr_Z·, which is known as Signal $II_{very\ fast}$ [33], decays within the response time of our EPR spectrometer. Thus, it is not observable during illumination. However, when the electron transfer from the oxygen-evolving Mn-cluster is inhibited for any reason, Tyr_Z· is reduced in a slower time-scale, and the spectrum becomes detectable [33] in the form of Signal II_{fast}. The spectrum in Fig. 4B shows that there is no increase of Signal II during continuous illumination in the PS II membranes (the only increase in the spectra is from P_{700}·). This shows that all Tyr_Z· is rapidly reduced, demonstrating that the oxygen evolving system is operational in the totally dominating fraction of the PS II centres of the preparation.

Another useful spectroscopic probe for the integrity of the oxygen-evolving system is the S_2 state multiline EPR signal that originates from the Mn-cluster [33]. In thylakoids from *Synechocystis* 6803 this spectrum cannot be observed due to the low PS II content (not shown). However, in the purified PS II membranes the multiline signal could easily be detected after illumination at 200 K. The recorded spectrum (Fig. 5) is very similar to the spectrum of PS II in higher plants, since it has the same hyperfine splitting and contains similar minor spectral components. However, the spectrum in *Synechocystis* 6803 is more resolved than the plant PS II spectrum, as seen from the better resolution of several of the shoulders on peaks in the central part of the spectrum (Fig. 5). This is similar to the situation in other cyanobacterial species [34].

Discussion

If *Synechocystis* 6803 is to be used as a model system for studies on the structure and function of the PS II complex, the experimental advantages of this organism with respect to site-directed mutagenesis have to be combined with the possibility of biochemical analysis. In particular, when it comes to functional studies of PS II, EPR spectroscopy is a crucial analytic tool. To fulfil this biochemical requirement it is necessary to obtain a PS II preparation with similar properties as the 'BBY'-preparation isolated from plant chloroplast [32]. Previously presented procedures for preparation of PS II from *Synechocystis* 6803 [22,35] require quite timeconsuming chromatographic procedures and give a poor yield of PS II material. In our hands, such procedures have resulted in preparations with low or no oxygen-evolving activity. The aim of this work has therefore been to develop a simple, high-yield PS II preparation that retains high rates of oxygen evolution, and of a purity that allows EPR analysis of various PS II components.

A direct approach involving a selective extraction of active PS II from the membrane by detergent solubilization was not successful. No conditions could be found that allowed for PS II extraction that did not

also result in destabilization of the oxygen evolving activity. Moreover, PS I was usually solubilized to a higher degree than PS II, as is often the case also for plant thylakoids [36]. Subsequent efforts to separate solubilized PS II and PS I by sucrose gradient centrifugation and liquid chromatography invariably led to substantial loss of oxygen evolution activity, and the resulting PS II preparation, even though of high purity, did not generate acceptable EPR signals.

Another approach involving selective detergent extraction of PS I from the thylakoids and subsequent isolation of the residual membranes by differential centrifugation resulted in a membrane fraction with an apparent 10–15-fold enrichment of functional PS II. A combination of dodecyl β-maltoside, Triton X-100 and the chaotropic salt $LiClO_4$ was used for selective extraction of PS I. The thylakoid suspension was pretreated with a low concentration of dodecyl β-maltoside prior to extraction with Triton X-100. Dodecyl β-maltoside probably has a stabilizing effect on the oxygen evolving capacity of PS II as reported earlier by Bowes et al. [37]. Without the pretreatment of dodecyl β-maltoside, much of the PS II activity was lost during the Triton X-100 treatment. Dodecyl β-maltoside also releases the phycobilisomes from the membrane surface, which may increase the overall accessibility to Triton X-100. The addition of $LiClO_4$ after detergent extraction had a stabilizing effect on PS II activity.

The PS II preparation is not a protein complex, but rather a population of membrane sheets where PS II is embedded in its natural lipid environment. In that respect, the present *Synechocystis* PS II preparation is organizationally analogous to the 'BBY'-preparation from plant chloroplasts [38].

Several redox-components on the oxidizing side of PS II have been well characterized by EPR spectroscopy. The possibility of studying these spectra (Tyr_Z, Tyr_D and the S-state multiline-signal) in the present preparation from *Synechocystis* 6803 should be of crucial future importance for the successful application of site-directed mutagenesis in structural and functional investigations of the photosynthetic oxygen-evolving system and the environment of Tyr_D and Tyr_Z in the PS II reaction centre.

In conclusion, the preparation should possess the same experimental potential for research on PS II in *Synechocystis* 6803 as the 'BBY'-preparation has had on studies of PS II from plants. In particular, it will allow EPR analysis of structure–function relationships after site-directed mutagenesis of various subunits in the reaction centre of PS II.

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References

1 Deisenhofer, J., Epp, O., Miki, M., Huber, R. and Michel, H. (1985) Nature 318, 618–624.
2 Nanba, O. and Satoh, K. (1987) Proc. Natl. Acad. Sci. USA 84, 109–112.
3 Barber, J., Chapman, D.J. and Telfer, A. (1987) FEBS Lett. 220, 67–73.
4 Trebst, A. (1986) Z. Naturforsch. 41C, 240–245.
5 Michel, H. and Deisenhofer, J. (1988) Biochemistry 27, 1–7.
6 Svensson, B., Vass, I., Cedergren, E. and Styring, S. (1990) EMBO J. 7, 2051–2059.
7 Nitschke, W. and Rutherford, A.W. (1991) Trends Biochem. Sci. 16, 241–245.
8 Diner, B.A., Nixon, P.J. and Farchaus, J.W. (1991) in Current Opinion in Structual Biology, Vol. 1, pp. 546–554, Current Biology Ltd. London.
9 Pakrasi, H.B. and Vermaas, W.F.J. (1992) in Molecular Approaches to Photosynthesis (Barber, J., ed.), Elsevier, Amsterdam, in press.
10 Rippka, R. (1972) Arch. Microbiol. 87, 93–98.
11 Grigorieva, G. and Shestakov, S. (1982) FEMS Microbiol. Lett. 13, 367–370.
12 Vermaas, W.F.J., Williams, J.G.K., Rutherford, A.W., Mathis, P. and Arntzen, C.J. (1986) Proc. Natl. Acad. Sci. USA 83, 9474–9477.
13 Pakrasi, H., Williams, J. and Arntzen, C.J. (1988) EMBO J. 7, 325–332.
14 Metz, J.G., Nixon, P.J., Rögner, M., Brudvig, G.W. and Diner, B.A. (1989) Biochemistry 20, 6960–6969.
15 Dzelzkalns, V.A. and Bogorad, L. (1989) Plant Physiol. 90, 617–623.
16 Jansson, C., Debus, R.J., Osiewacs, H.D., Gurevitz, M. and McIntosh, L. (1987) Plant Physiol. 85, 1021–1025.
17 Mayes, S.R., Cook, K.M., Self, S.J., Zhang, Z.H. and Barber, J. (1991) Biochim. Biophys. Acta 1060, 1–12.
18 Burnap, R.L. and Sherman, L.A. (1991) Biochemistry 30, 440–446.
19 Astier, C., Styring, S., Maison-Petri, B. and Etienne, A.-L. (1986) Photobiochem. Photobiophys. 11, 37–47.
20 Williams, J.G.K. (1988) Methods Enzymol. 167, 766–778.
21 Nilsson, F., Andersson, B. and Jansson, C. (1990) Plant Mol. Biol. 14, 1051–1054.
22 Burnap, R., Koike, H., Sotiropoulou, G., Sherman, L.A. and Inoue, Y. (1989) Photosynth. Res. 22, 123–130.
23 Hiyama, T. and Ke, B. (1972) Biochim. Biophys. Acta 267, 169–171.
24 Laemmli, U.K. (1970) Nature 227, 680–685.
25 Ljungberg, U., Åkerlund, H.-E. and Andersson, B. (1986) Eur. J. Biochem. 158, 477–482.
26 Sètif, P., Ikegami, I and Biggins, J. (1987) Biochim. Biophys. Acta 894, 146–156.
27 Rögner, M., Nixon, P.J. and Diner, B.A. (1990) J. Biol. Chem. 265, 6189–6196.
28 Butler, W.L. (1978) Annu. Rev. Plant Physiol. 29, 345–378.
29 Golbeck, J.H. and Bryant, D.A. (1991) in Current Topics in Bioenergetics (Lee, C.P., ed.), Vol. 16, pp. 83–177, Academic Press, San Diego.
30 Debus, R.J., Barry, B.A., Babcock, G.T. and McIntosh, L. (1988) Proc. Natl. Acad. Sci. USA 85, 427–430.
31 Vermaas, W., Rutherford, A.W. and Hansson, Ö. (1988) Proc. Natl. Acad. Sci. USA 85, 8477–8481.

32 Berthold, D.A., Babcock, G.T. and Yocum, C.F. (1981) FEBS Lett. 134, 231–324
33 Miller, A.-F. and Brudvig, G.W. (1991) Biochim. Biophys. Acta 1056, 1–18.
34 Aasa, R., Andréasson, L.-E., Lagenfelt, G. and Vänngård, T. (1987) FEBS Lett. 221, 245–248.
35 Noren, G.H., Boerner, R.J. and Barry, B.A. (1991) Biochemistry 30, 3943–3950.
36 Andersson, B. and Anderson, J.M. (1985) in Modern Methods in Plant Analysis (Linskens, H.F. and Jackson, J.F., eds.), Vol. I, pp. 231–258, Springer, Berlin.
37 Bowes, J.M., Stewart, A.C. and Bendall, D.S. (1983) Biochim. Biophys. Acta 725, 210–219.
38 Dunahay, T.G., Staehelin, L.A., Seibert, M., Olijivere, P.D. and Berg, S.P. (1984) Biochim. Biophys. Acta 764, 179–193.