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PURIFICATION OF PHOTOSYSTEM II PARTICLES FROM *PHORMIDIUM LAMINOSUM* USING THE DETERGENT DODECYL-β-D-MALTOSIDE

PROPERTIES OF THE PURIFIED COMPLEX

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The purification and properties of a new oxygen-evolving Photosystem (PS) II particle from the thermophilic blue-green alga *Phormidium laminosum* are described. The activity of the lauryldimethylamine *N*-oxide PS II-enriched supernatant described previously (Stewart, A.C. and Bendall, D.S. (1979) FEBS Lett. 107, 308–312) was found to be stabilized for several days at 4°C by the addition of a second detergent, dodecyl-β-D-maltoside (lauryl maltoside). The lauryl maltoside/lauryldimethylamine *N*-oxide extract could be fractionated by sucrose density gradient centrifugation. Very high rates of oxygen evolution, typically 1900–2400 μmol O_2/mg chlorophyll *a* per h at pH 7 with dimethylbenzoquinone and ferricyanide as acceptors, were observed for the lowest green band from the gradient. This fraction contained cytochromes *b*-559 (high-potential) and *c*-549, but was completely devoid of P-700 and cytochromes *b*-563 and *f*. The purified oxygen-evolving particles comprised seven major polypeptides (M_r 58900, 52400, 43200, 33900, 30000, 16000 and 15000) and approximately five minor polypeptides. The particles contained 3–4 Mn atoms per reaction centre and had a chlorophyll antenna of approx. 50 chlorophyll *a*. The fast phase of fluorescence induction curves in the presence of hydroxylamine and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) could be described by an exponential, suggesting that no energy transfer was occurring between the PS II units responsible for this phase. Comparison of the area above the fluorescence induction curves in the absence and presence of DCMU suggested an acceptor pool size of 2–3 equivalents per centre.

Introduction

An understanding of the mechanism of the photosynthetic water-splitting reaction catalysed by PS II of higher plants and algae is now most likely to come from the study of an isolated photosystem complex which retains the capacity to evolve oxygen but consists only of the essential components. Recently, the first PS II particles to show higher rates of light-induced oxygen evolution per chlorophyll than the original membranes

Abbreviations: Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DMBQ, 2,6-dimethyl-1,4-benzoquinone; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; LDAO, dodecyldimethylamine *N*-oxide (lauryldimethylamine *N*-oxide); lauryl maltoside, dodecyl-β-D-maltoside; menadione, 2-methyl-1,4-naphthoquinone; Mes, 2-(*N*-morpholino)ethanesulphonic acid; Mops, 3-(*N*-morpholino)propanesulphonic acid; P-680, primary chlorophyll electron donor of Photosystem II; P-700, primary chlorophyll electron donor of Photosystem I; PS, photosystem; PMSF, phenylmethylsulphonyl fluoride; Q, primary quinone acceptor of Photosystem II; TMBZ, 3,3′,5,5′-tetramethylbenzidine; Tricine, *N*-tris(hydroxymethyl)methylglycine.

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were described by Stewart and Bendall [1,2]; this preparation was obtained from a thermophilic blue-green alga, *Phormidium laminosum*. Since then a number of PS II preparations showing some capacity for oxygen evolution have been isolated, both from higher plants [2–5] and from other blue-green algae [6]. Although several of these preparations appear promising for studies on the mechanism of oxygen evolution, none completely fulfils the criterion of maximal activity combined with reasonable purity.

The preparation from *P. laminosum* was extracted from the photosynthetic membranes with the detergent LDAO and was enriched 5–6-fold in oxygen-evolving activity on a chlorophyll basis [1]. It was substantially depleted of P-700, had a chlorophyll antenna of 40–60 per reaction centre [2,7] and had a simpler acceptor system than that of higher plants [8], although the latter two properties were probably also characteristic of the original membranes [9]. Nevertheless, the preparation was not chemically pure and contained a large number of polypeptides which are not constituents of PS II, for example, cytochromes *b*-563 and *f* [2]. On the other hand, although the preparations from higher plants contain fewer polypeptides, they retain large amounts of the light-harvesting Chl *a*/*b*-protein complex and thus have a relatively large photosynthetic unit size (200–250 Chl per centre) [3].

In this paper we report a purification of the *P. laminosum* particle which has been achieved without substantial loss of activity. The procedure involved treatment of the initial PS II-enriched LDAO extract with a second detergent, lauryl maltoside, followed by fractionation on a sucrose density gradient. This treatment led to the removal of many contaminating proteins including the residual PS I and cytochromes *b*-563 and *f*, and also stabilized the activity of the particles.

Materials and Methods

Preparation of PS II particles. Thylakoid membranes were isolated from *P. laminosum* strain OH-1-p. Cl 1 [10] as described previously [1,11], except that bovine serum albumin (Sigma, fraction V, 2% (w/v)) was included in the medium during lysozyme treatment, and bovine serum albumin (0.5%) plus DNAase (BDH, 5 μg/ml) were included in the hypo-osmotic buffer used to lyse the spheroplasts.

Membranes (1.0 mg Chl *a*/ml) were incubated with 0.38% (v/v) LDAO for 40 min at 0°C in the dark in buffer C containing 25% (v/v) glycerol, 10 mM $MgCl_2$, 10 mM Hepes-NaOH and 5 mM sodium phosphate, pH 7.5, plus 1 mM PMSF. The mixture was centrifuged at $100000 \times g$ for 1 h. The supernatant was carefully decanted and lauryl maltoside (Calbiochem) was added immediately to give a lauryl maltoside/Chl ratio of 40 : 1 (w/w). 6 ml of treated supernatant were layered onto 74 ml of a 15–35% (w/v) continuous sucrose gradient in buffer containing 10% (v/v) glycerol, 10 mM $MgCl_2$, 10 mM Hepes-NaOH, pH 7.5, 1 mM PMSF and sufficient lauryl maltoside to give a total lauryl maltoside/Chl ratio of 20 : 1 (w/w). The gradient was centrifuged for 20 h at 44000 rpm ($225000 \times g_{max}$) in a Beckman 45Ti rotor. Fractions, starting from the lowest one, were removed from the gradient using a syringe with a flat-tipped needle.

Chlorophyll and protein assays. Chl *a* was assayed as described by Arnon et al. [12]. Protein was assayed by the method of Lowry et al. [13]. Sucrose was removed from the samples before protein assays, by chromatography on a Sephadex G-25 column equilibrated with 20 mM Hepes-NaOH, pH 7.5. Bovine serum albumin was used as the protein standard.

Assays of electron transfer components. Total manganese was measured by flameless atomic absorption spectrometry [2] in samples acidified with 0.1 M HCl and diluted 5–20-fold with low-conductivity 'milli-Q' water from a Millipore purification system (Millipore Corp., Bedford, MA, U.S.A.). Values obtained for buffer blanks (containing buffer components only) were subtracted from the readings. Standards were prepared by dilution of a solution of standard Mn (BDH, manganous nitrate for atomic absorption spectroscopy).

Cytochromes and P-700 were determined from chemical reduced-minus-oxidized difference spectra as described previously [2]. Spectra were recorded on a Cary 219 spectrophotometer with automatic baseline correction.

SDS-polyacrylamide gel electrophoresis. Electro-

phoresis was carried out using the buffer system of Chua [16]. Gels were run for 16 h at 90 V. Two methods of sample preparation were used:

(a) When the gel was to be stained with Coomassie blue (BDH PAGE blue 83) as in Ref. 16, sucrose was removed from the sucrose gradient fractions by chromatography on a Sephadex G25 column equilibrated with buffer C. The samples were then concentrated in an Amicon ultrafiltration cell (YM 30 membrane) to a final chlorophyll concentration of approx. 0.1 mg/ml. Unfractionated membranes (1.0 mg Chl *a*/ml) were pre-treated for 40 min with 0.4% LDAO then diluted 5-fold with buffer C. Samples of PS I particles [1] and of the lauryl maltoside-treated LDAO supernatant were diluted in buffer C to 0.4 and 0.1 mg Chl *a*/ml, respectively. 25 μl of each sample prepared as described above were treated with 25 μl of solubilizing buffer containing 4% SDS, 0.04% bromophenol blue, 50 mM dithiothreitol and 0.1 M Hepes-NaOH, pH 7.5. Samples were incubated for 30 min at 37°C before application to a 7.5–15% acrylamide gradient gel, with a 5% stacking gel.

(b) When the gel was to be stained with TMBZ-H_2O_2 for detection of cytochromes as in Ref. 17, 500-μl samples of the sucrose gradient fractions were treated at 0°C with 500 μl of 10% trichloroacetic acid. After 1 h the precipitated protein was collected by centrifuging, the pellets washed with 0.5 ml acetone, dried under a stream of nitrogen and finally resuspended in 35 μl SDS-containing solubilizing buffer as in Ref. 16. Samples were incubated for 1 h at 20°C, then applied to a 15% acrylamide gel with a 5% stacking gel. This method allowed much higher sample loadings than method a, though cytochrome *b*-563 was not always well resolved (see Results). The method also resulted in a change in apparent M_r for cytochrome *f* from 28000 to 37000. A similar change in M_r for cytochrome *f* from *P. laminosum* occurred if samples were heated to 60°C before electrophoresis [2]. There was no change in apparent M_r for cytochromes *b*-563 and *c*-549.

Fluorescence. Fluorescence was measured using an Applied Photophysics single-beam spectrophotometer apparatus. Excitation was provided by a 15 V quartz-halogen lamp screened by an Oriel broad-band filter ($\lambda_{max} = 449$ nm) plus a heat filter, and fluorescence at 685 nm was monitored at right angles by a photomultiplier screened by a Balzers B-40 685 nm interference filter. The amplified signal from the photomultiplier was fed into a Datalab (DL 902) transient recorder and then plotted directly onto an X-Y plotter.

Rates of electron transfer. Oxygen evolution was assayed at 25°C using a Hansatech oxygen electrode as described previously [1,2,11]. The electrode was calibrated for the reaction buffers by the method of Robinson and Cooper [14]. The artificial electron acceptors used were 6 mM DMBQ, 6 mM DMBQ plus 3 mM potassium ferricyanide, and 10 mM ferricyanide alone. The concentrations of DMBQ and ferricyanide were higher than those used in previous studies, since it was found that 1 mM DMBQ and 2 mM ferricyanide [1,2,11] were not saturating. At pH 7.3 in the presence of 6 mM DMBQ plus 3 mM ferricyanide, unfractionated membranes of *P. laminosum* showed oxygen evolution rates of 350–430 μmol O_2/mg Chl *a* per h.

Phycobilisomes.

P. laminosum phycobilisomes were prepared as described in Ref. 15 except that cell breakage was not carried out by French pressure cell treatment. Instead, spheroplasts prepared by lysozyme treatment as described above were treated directly with 2% (w/v) Triton X-100 in 0.75 M potassium phosphate buffer, pH 6.9.

Results

The effect of lauryl maltoside on the LDAO supernatant

Treatment of membrane fragments from *P. laminosum* with the detergent LDAO results in the selective solubilization of PS II, which appears enriched in the supernatant following centrifugation at 100000 × *g* for 1 h [1]. Fig. 1 shows the effect of treating this PS II-enriched supernatant with various concentrations of a second detergent, lauryl maltoside. Increasing concentrations of lauryl maltoside enhanced the stability of oxygen evolution in samples incubated in the dark at 4°C. At a detergent/Chl ratio of 40 : 1 (w/w), the rate of oxygen evolution was approx. 80% of the original 1800 μmol O_2/mg Chl *a* per h after 40 h of incubation, compared with approx. 35% in the untreated sample. The related alkyl glycoside, oc-

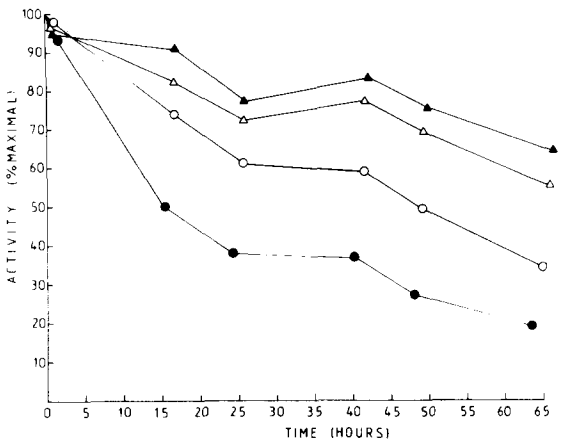

Fig. 1. Effect of lauryl maltoside on the stability of oxygen-evolving activity of the PS II-enriched LDAO supernatant from *P. laminosum*. Membrane fragments were treated with LDAO as described in Materials and Methods. Lauryl maltoside was added at time = 0 to the LDAO supernatant. Measurements of O_2 evolution with 6 mM DMBQ plus 3 mM ferricyanide were performed as described in Materials and Methods. Activities are expressed as percentages of the initial activity of the LDAO supernatant (1900 μmol O_2/mg Chl *a* per h). Ratios of lauryl maltoside to Chl as follows: (●) 0:1, (○) 5:1, (△) 20:1, (▲) 40:1.

tylglucoside, was ineffective in stabilizing activity. Lauryl maltoside has also been reported to stabilize preparations of rhodopsin [18], cytochrome oxidase [19] and photosynthetic reaction centres isolated from *Rhodopseudomonas sphaeroides* with LDAO [20]. In the last case, lauryl maltoside was claimed to prevent pheophytinization of the bacteriochlorophyll.

The stabilization of the activity of PS II in the presence of lauryl maltoside (Fig. 1) suggested that under these conditions it might be possible to fractionate the preparation without significant loss of activity, whereas this had not been possible previously [2,21]. Lauryl maltoside was ineffective as a substitute for LDAO in the initial selective solubilization of PS II from *P. laminosum* thylakoids, so a fractionation procedure involving sequential use of the two detergents was devised.

Fractionation of the lauryl maltoside-treated LDAO supernatant

A LDAO supernatant treated with lauryl maltoside (lauryl maltoside/Chl = 40:1, w/w) was subjected to sucrose density gradient centrifugation on a 15–35% (w/v) gradient containing lauryl maltoside at a ratio of detergent/Chl = 20:1 (w/w) (see Materials and Methods). After centrifugation, six pigmented bands were resolved. Absorption spectra (not shown) revealed that bands 1 (orange) and 2 (pale green) at the top of the gradient were rich in carotenoids and also contained some detergent-solubilized chlorophyll. The bulk of the blue phycobiliprotein, phycocyanin, was in bands 2–4. Band 5, which was bluish-green, contained a large amount of allophycocyanin, and there was also a small amount of this pigment in the main chlorophyll-containing band, band 6. The chlorophyll peak was at 671.5 nm in band 6.

Activity. Approx. 85% of the initial total oxygen-evolving activity was recovered from the sucrose gradient and of that, 98% was found in fractions 4–6. The most active fraction, 6, was enriched approx. 20% in oxygen evolution per chlorophyll and 2-fold in oxygen evolution per protein compared with the initial lauryl maltoside-treated LDAO supernatant (Table I). The activity was inhibited 90–98% by 10 μM DCMU under the conditions of Table I (but see also below). The rate of oxygen evolution measured in fraction 6 was significantly higher than in any other reported preparation of PS II. This high activity also showed substantial stability, declining by only 20% over 20 h and 50% over 40 h at 4°C.

P-700. Fraction 6 was found to be devoid of P-700 in reduced-minus-oxidized difference spectra (Table II), but small amounts of P-700 were detectable in fractions 1–5. These results were confirmed by lithium dodecyl sulphate polyacrylamide gel electrophoresis at 4°C [22]. The PS II chlorophyll-protein complex [23] was clearly visible in fractions 2–6, and was the only green band in fraction 6, while fractions 2–4 also contained a faint green band corresponding to the PS I chlorophyll-protein complex (results not shown).

Cytochromes. The distribution of cytochromes in the gradient was determined by chemical difference spectra and by TMBZ-H_2O_2 (haem) staining of SDS-polyacrylamide gels (Fig. 2). Cytochromes *b*-563 and *f* were concentrated in fractions 2–4 on TMBZ-H_2O_2-stained gels and were com-

TABLE I

ELECTRON-TRANSPORT ACTIVITY OF *P. LAMINOSUM* PS II PARTICLES AFTER FRACTIONATION ON A LAURYL MALTOSIDE-CONTAINING SUCROSE DENSITY GRADIENT

The preparation of an LDAO supernatant, treatment with lauryl maltoside and sucrose density gradient centrifugation were carried out as described in Materials and Methods. Fractions 1–6 were recovered from the gradient as shown in Fig. 2. Assays were carried out in 10 mM Hepes-NaOH buffer, pH 7.3, containing 25% glycerol and 10 mM $MgCl_2$. Concentrations of other reagents were as listed in Materials and Methods.

Fraction No.	Chlorophyll (% of total)	Rate of O_2 evolution ($H_2O \rightarrow$ DMBQ + ferricyanide)		
		(μmol O_2/mg Chl *a* per h)		(μmol O_2/mg protein per h)
		− DCMU	+ DCMU	
1	4	0	–	0
2	12	0	–	0
3	8	385	0	8.5
4	5	903	52	19.0
5	27	1343	112	31.6
6	51	2288	34	84.5
LDAO supernatant	100	1860	0	42.6
Lauryl maltoside-treated supernatant	100	1884	0	43.1

pletely absent from fraction 6 (Fig. 2). Fraction 6 contained only one haem-staining band, at M_r 14700, which was attributed to the low-potential cytochrome *c*-549 [2]. This cytochrome was also present in the other fractions. There was no evidence for a haem-staining band in Fig. 2 that could be attributed to cytochrome *b*-559. However, this cytochrome could be detected in difference spectra for all of the fractions, and was particularly concentrated in fractions 4–6 (Table II), in which an increasing proportion of the cytochrome was in a high-potential form (reducible by methylhydroquinone at pH 6.5). Table II gives the proportions for one preparation, but considerable variability was observed; in some preparations more than 90% of the cytochrome was methylhy-

TABLE II

P-700, CYTOCHROME *f* AND CYTOCHROME *b*-559 CONTENT OF *P. LAMINOSUM* PS II PARTICLES AFTER FRACTIONATION ON A LAURYL MALTOSIDE-CONTAINING SUCROSE DENSITY GRADIENT

P-700 and cytochrome *f* were assayed by recording chemical reduced-minus-oxidized difference spectra as in Ref. 11. High-potential and total cytochrome *b*-559 were estimated, respectively, from 2 mM methylhydroquinone-minus-2 mM ferricyanide and 1 mM menadiol-minus-2 mM ferricyanide difference spectra at pH 6.5.

Fraction No.	P-700 (nmol/mg Chl *a*)	Cytochrome *f* (nmol/mg Chl *a*)	Cytochrome *b*-559 (nmol/mg Chl *a*)	% of cytochrome *b*-559 in high-potential form
1	1.5	8.3	8.5	0
2	1.4	18	11	0
3	1.7	13	14	8
4	1.9	3.8	16	22
5	0.90	2.2	20	65
6	0	0	22	78
Lauryl maltoside-treated supernatant	0.96	4.3	20	90

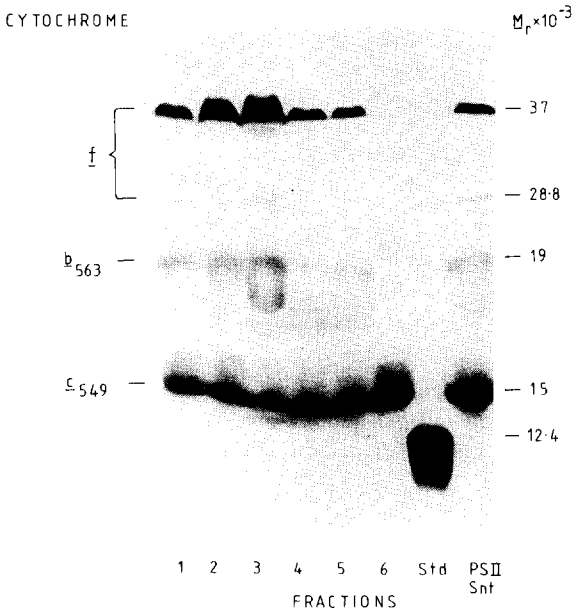

Fig. 2. Haem-containing polypeptides of PS II-enriched LDAO supernatant (PSII Snt) and of fractions 1–6 from the sucrose density gradient. Samples were prepared for electrophoresis as described in Materials and Methods. The gel was stained with TMBZ-H_2O_2 as in Ref. 17. Mammalian cytochrome *c* (M_r 12400) was used as the standard (Std).

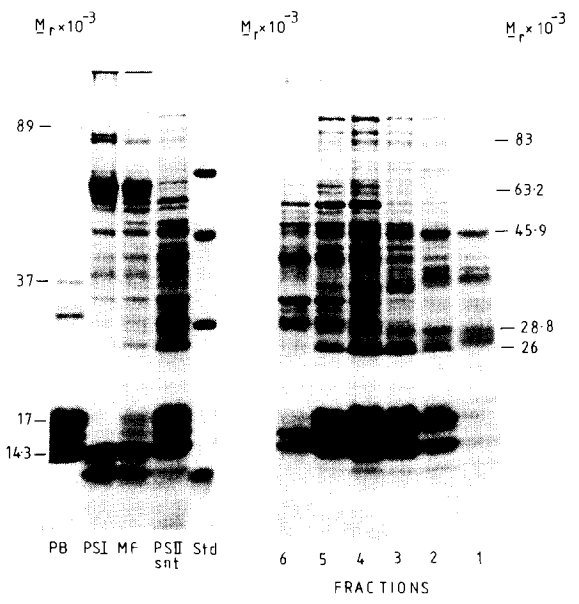

Fig. 3. Polypeptide profiles of *P. laminosum* membranes (MF), PS I particles (PSI), PS II-enriched LDAO supernatant (PSII Snt), phycobilisomes (PB) and fractions 1–6 from the sucrose density gradient. Samples were prepared and electrophoresis carried out as described in Materials and Methods. M_r standards (Std) were bovine serum albumin (68000), ovalbumin (43000), carbonic anhydrase (30000) and cytochrome *c* (12400). The gel was stained with Coomassie Blue.

droquinone reducible, in others only 40% was reduced. There was no apparent correlation between the capacity to evolve oxygen and the proportion of the cytochrome in the high-potential form.

Manganese. All the fractions contained large amounts of Mn; the amount of Mn relative to chlorophyll was particularly high in fractions 1 and 6. The amount of Mn in fraction 6 compared with the estimated antenna size for this fraction (see below) suggested approx. 4 Mn per reaction centre.

Polypeptides. The most active fraction from the sucrose gradient, fraction 6, had a substantially simpler polypeptide profile than the initial detergent extract (Fig. 3). Several of the contaminating polypeptides removed during the fractionation correspond with polypeptides of PS I, phycobilisomes and the cytochrome *b*-563-*f* complex (Figs. 2 and 3). Fraction 6 contained seven major Coomassie blue-stained bands (M_r 58900, 52400, 43200, 33900, 30000, 16000 and 15000) and minor bands at M_r 120000, 57500, 54100, 50800 and 32900. Some of these bands may be tentatively identified. Allophycocyanin contributed bands at M_r 15000 and 16000 (these bands were purple in unstained gels) while cytochrome *c*-549 (M_r 15000) co-migrated with the lower allophycocyanin band. The band at M_r 58900, which was enriched in the less active fractions 4 and 5, may be a contaminant, perhaps a subunit of coupling factor CF_1 [24]. The PS II reaction centre chlorophyll-protein of *P. laminosum* has been found to contain two bands in the region 40000–42000 and 46000–50000 [23]. A weakly stained band at M_r 32900 may be the herbicide-binding protein [25–27], while the stronger band at M_r 33900 may correspond to a protein that has been identified as a component on the oxidising side of PS II [5,28,29].

Properties of fraction 6: Purified oxygen-evolving particles

pH dependence and DCMU sensitivity of oxygen evolution. The highest rates of oxygen evolution by the particles (1900–2400 μmol O_2/mg Chl *a* per h), were observed between pH 6.7 and pH 7.2 with 6 mM DMBQ plus 3 mM ferricyanide as the acceptor system (Fig. 4). When ferricyanide alone was the acceptor, a much lower pH optimum was observed, at pH 5.0, and a high concentration of ferricyanide (10 mM) was required to saturate the reaction. A possible explanation for this effect is that Q became more accessible to the charged acceptor, ferricyanide, when negative surface charges on the particle were neutralized at low pH [30]. This explanation is supported by the fact that electron transport in the presence of ferricyanide became increasingly insensitive to DCMU at low pH (Fig. 5), indicating that ferricyanide was able to accept electrons before the DCMU block. Similar results were obtained for unfractionated membranes, but the degree of insensitivity to DCMU was less than in the particles. For example at pH 5.2, 6.0 and 6.8 in the presence of 10 mM ferricyanide, oxygen evolution in membranes was, respectively, 25, 10 and less than 5% insensitive to DCMU, compared with approx. 65, 30 and 20% insensitivity for the particles. In contrast, oxygen evolution with DMBQ alone as acceptor was almost completely inhibited by DCMU throughout the range pH 5–8, both in the particles (Fig. 5) and in unfractionated membranes (not shown).

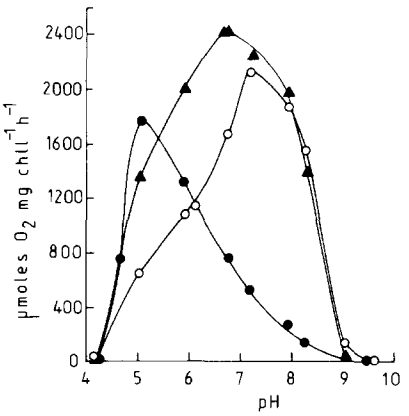

Fig. 4. Effect of pH and electron acceptor on oxygen evolution in purified oxygen-evolving particles. Particles were suspended at 7.4 μg Chl *a*/ml in 25% glycerol, 10 mM $MgCl_2$ plus one of the following buffers adjusted to the appropriate pH: 50 mM glycine (pH 9), 50 mM Tricine (pH 8–9), 50 mM Hepes (pH 7.5–8), 50 mM Mops (pH 7), 50 mM Mes (pH 5–6), 50 mM glycylglycine (pH 5). Light-induced oxygen evolution was measured as described in Materials and Methods, with the following acceptors: (○) 6 mM DMBQ, (●) 10 mM ferricyanide, (▲) 6 mM DMBQ+3 mM ferricyanide.

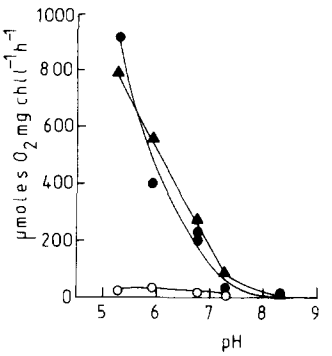

Fig. 5. Effect of pH and electron acceptor on DCMU-insensitive oxygen evolution in purified oxygen-evolving particles. Conditions as for Fig. 6, except measurements were performed in the presence of 10 μM DCMU. (○) 6 mM DMBQ, (●) 10 mM ferricyanide, (▲) 6 mM DMBQ+3 mM ferricyanide.

Effect of calcium. There have been various reports of stimulation of ferricyanide-supported oxygen evolution in preparations from blue-green algae by calcium [6,31,32]. However, in the *P. laminosum* PS II particles, stimulation by 25 mM $CaCl_2$ (10–40% in different preparations) was only observed at alkaline pH with a high concentration of ferricyanide (10 mM) as acceptor, suggesting that the effect was on the reducing rather than the oxidising side of PS II (see also Ref. 33). This effect was not produced by addition of either 25 mM $MgCl_2$ or 125 mM NaCl, nor was it antagonised by the calmodulin antagonist chlorpromazine. This result does not necessarily preclude a role for calcium on the oxidizing side of PS II. There is recent evidence that calcium does have such a role [34], but, as in the case of the chloride requirement for oxygen evolution [35], some disruption of the native PS II complex and more rigorous depletion of its endogenous ionic content may be necessary to demonstrate the effect.

Fluorescence. Fluorescence is emitted from the chlorophyll antenna associated with PS II reaction centres in inverse proportion to the yield of photo-

chemistry; thus, the yield of fluorescence increases during the light-induced reduction of the primary acceptor Q [36]. Illumination of the particles produced a high yield of variable fluorescence, indicative of an intact and functional light-harvesting chlorophyll antenna. In the presence of DCMU and hydroxylamine the maximal yield (F_t) divided by the initial yield (F_0) was 4.5–5.0, slightly higher than previously observed in unpurified *P. laminosum* PS II particles [7,9]. In addition, the total yield of fluorescence per chlorophyll in the purified particles was higher, consistent with the removal from the present preparation of some non-fluorescent chlorophyll associated with PS I.

The area above fluorescence induction curves is proportional to the number of electron acceptors [37]. The area above the fluorescence induction curve in the particles, measured in the presence of hydroxylamine to prevent back-reactions [38], was 2–3-times larger in the absence than in the presence of DCMU. Assuming one primary acceptor for the particles before the site of DCMU inhibition, as in Ref. 8, this suggests an acceptor pool of 2–3 equivalents in the absence of DCMU. Thus, most of the plastoquinone pool, normally comprising approx. 10 equivalents in particles prepared using LDAO alone [1], had been extracted by lauryl maltoside.

Fig. 6 shows the normalised variable component of fluorescence measured in the presence of hydroxylamine and DCMU in *P. laminosum* LDAO particles (open circles) and in the purified particles (closed circles). Both curves were clearly biphasic; the origin of the slow phase of the induction has been discussed previously [7]. The fast phase was sigmoidal for the LDAO particles, and

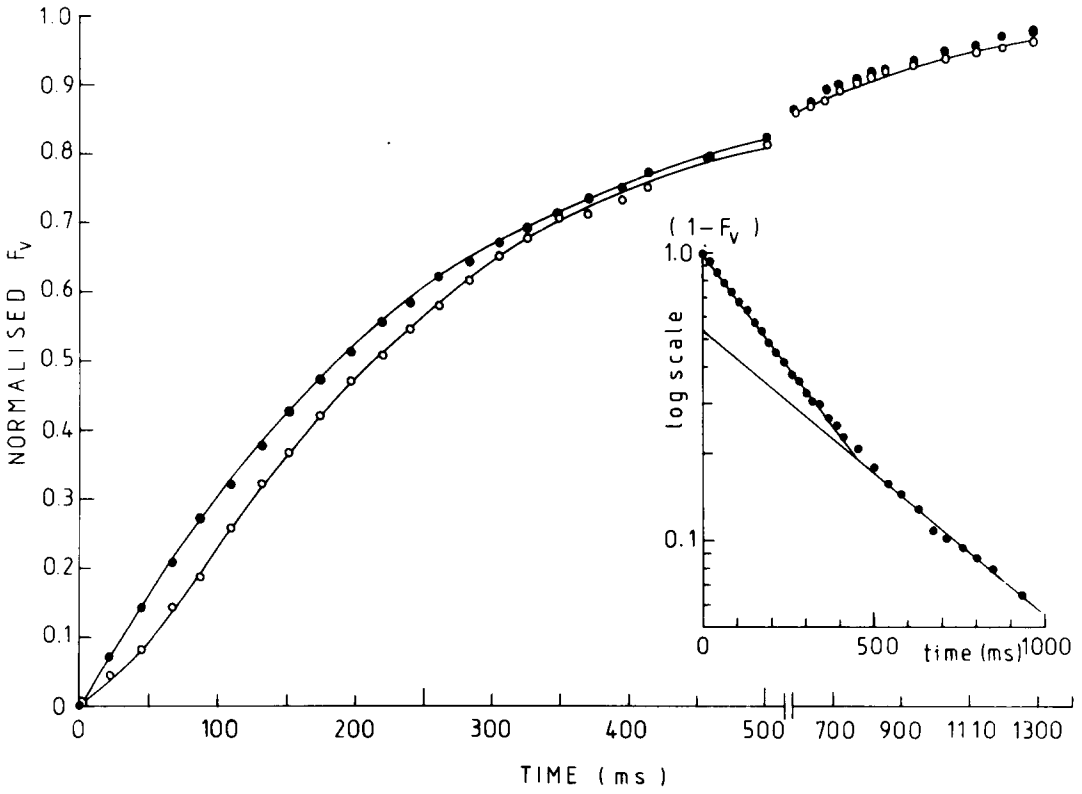

Fig. 6. Variable fluorescence in purified oxygen-evolving particles and LDAO-PS II particles. Fluorescence induction curves were measured as described in Materials and Methods. LDAO-PSII particles (prepared as in Ref. 1) and purified oxygen-evolving particles were dark adapted for 20 min in buffer C at 5 μg Chl *a*/ml. 10 mM hydroxylamine and 10 μM DCMU were added before measurement. The variable component of the fluorescence (F_v) has been normalised. Inset shows log plot of data obtained with purified oxygen-evolving particles. (●) Purified oxygen-evolving particles, (○) LDAO-PS II particles.

this has been attributed to the occurrence of energy transfer between several photosynthetic units in this preparation [7,9,39]. In contrast, the fast phase of the induction in the purified particles was exponential (Fig. 6, inset) which suggested that energy transfer between the centres was not occurring. The half-times for the variable fluorescence rise in the purified particles (190 ms) and LDAO particles (210 ms) under identical conditions (Fig. 6) were sufficiently similar to suggest that the size of the chlorophyll antenna, approx. 50 per centre, was the same in both preparations [2,7,9].

Discussion

The *P. laminosum* purified oxygen-evolving particles, which fulfil the criteria of high activity, stability and reasonable purity, promise to be very useful in studies on the mechanism of oxygen evolution. In the presence of saturating concentrations of the appropriate electron acceptors, the particles were capable of extremely high rates of oxygen evolution (at least 1700 μmol O_2/mg Chl *a* per h) over a wide range of pH from 5.0 to 8.0. We suggest that the acid pH optimum (pH 5–6) quoted for Hill reaction rates in some reports on oxygen-evolving particles [3,21,40] may arise from the use of ferricyanide as the predominant acceptor, rather than reflecting a property of the oxygen-evolving enzyme itself.

The stabilization of activity in the particles in the presence of lauryl maltoside is not fully understood at present, but may involve the replacement by lauryl maltoside of much of the LDAO, natural lipid and water surrounding the PS II complex, with the formation of mixed lauryl maltoside/protein micelles [18]. The suggestion that lipid was removed from the complex is supported by the finding that the purified particles had a smaller acceptor pool size than the *P. laminosum* PS II particles prepared with LDAO alone, indicating removal of bulk plastoquinone. The lack of energy transfer between the antenna chlorophylls of different PS II centres in the purified particles could also be interpreted as indicating that the centres were dispersed in detergent micelles.

Fractionation by lauryl maltoside treatment and sucrose density gradient centrifugation removed all the residual PS I, cytochromes *b*-563 and *f* and a number of unidentified polypeptides from the initial PS II-enriched supernatant, without substantially affecting activity. The resulting particles may contain few significant contaminants, the main three being the low-potential cytochrome *c*-549, some allophycocyanin and an unidentified component of M_r 58900. The simple polypeptide profile of the particles makes it possible to begin to assign specific roles to specific components. Four polypeptides, of M_r 52400, 43200, 33900 and 30000, appear to be strong contenders as components of the oxygen-evolving enzyme. No evidence was found for a component of M_r 23000 which might correspond to the protein found to reconstitute oxygen evolution in salt-washed inside-out vesicles from spinach [41]; however, we do not rule out the possibility that a corresponding protein in our preparation may migrate differently (perhaps in an aggregated form) during electrophoresis. As yet we have not assigned any of the bands observed on SDS-polyacrylamide gels to cytochrome *b*-559. There have been reports that cytochrome *b*-559 has an M_r of approx. 10000 [42], but the particles did not contain a major constituent in that region.

The substantial purification of the *P. laminosum* PS II particles described in this paper may have been achieved at the cost of some disruption of the native conformation of the PS II complex. Thus, the decreased DCMU sensitivity of ferricyanide reduction indicates partial exposure of Q, which is apparently more completely shielded by the DCMU-binding protein in unfractionated membranes, especially at neutral pH. The partial conversion of high-potential cytochrome *b*-559 to a lower-potential form also indicates some modification of the PS II complex. However, neither of these factors appeared to affect the activity of the oxygen-evolving enzyme itself, and the retention of 4 Mn per PS II centre as well as of the full chlorophyll antenna of the reaction centre complex are indicators of functional integrity.

Note added in proof (Received September 22nd, 1983)

There have been recent reports of the other algal PS II preparations showing similarly high rates of O_2 evolution [43,44].

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