Photosystem II particles largely depleted in the two intrinsic polypeptides in the 30 kDa region from *Synechococcus* sp.

Identification of a subunit which carries the photosystem II reaction center

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Treatment of oxygen-evolving photosystem II particles isolated from the thermophilic cyanobacterium, *Synechococcus* sp. with β-octylglucoside specifically solubilized two intrinsic polypeptides of 31 and 28 kDa from the particles. The removal of the two polypeptides had no significant effect on photoreduction of the bound quinone acceptor Q_A. The results indicate that the 31 and 28 kDa polypeptides are not essential for the primary charge separation and subsequent reduction of Q_A and hence support the view that the reaction center and Q_A are located on the chlorophyll-carrying 47 kDa subunit.

Q_A *D-2 protein* *Reaction center subunit* *Photosystem II* (Synechococcus *sp.*)

1. INTRODUCTION

The primary photochemistry of PS II is electron transfer from the excited donor chlorophyll P680 to the acceptor pheophytin, and the separated charges are stabilized by oxidation of the reduced pheophytin by the bound plastoquinone Q_A and by reduction of the oxidized P680 by the electron donor Z (review [1]). Electron transport from Z to Q_A takes place in the reaction center complex which consists of five subunits of 51–44, 44–40, 34–31, 32–28 and 10–7 kDa regions [2–4]. The two large subunits carry chlorophyll *a* [2,3] and the smallest one is the apoprotein of cytochrome *b*-559 [2]. Of the two polypeptides in the 30 kDa region, the smaller one (as judged from its mobility in SDS-polyacrylamide gels containing urea) is the herbicide-binding protein [2,5], but the function of the other is not known.

Currently, there are two hypotheses as to which subunit of the PS II reaction center complex carries the reaction center. There is ample evidence suggesting that the 51–44 kDa subunit is the site of the primary photochemistry of PS II. (i) Chlorophyll-protein complexes containing only the 47 kDa subunit emit a fluorescence band at 695 nm at 77 K [3,6,7], which is postulated to originate from pheophytin [8]. (ii) Distributions of DCIP photoreducing activity and photo-reducible pheophytin parallel that of the 51–44 kDa subunit among PS II fractions separated from spinach [9] and *Chlamydomonas* mutant [10], respectively. (iii) A *Synechococcus* PS II reaction center complex which contains 47, 31, 28 and 9 kDa subunits but totally lacks the 40 kDa apoprotein shows photoreduction of pheophytin and Q_A as well as high rate and quantum yield of DCIP photoreduction, whereas no PS II photochemistry was

Abbreviations: PS, photosystem; DCIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea

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detected in chlorophyll-protein containing only the 40 kDa subunit [11,12]. A maize PS II preparation which is totally deficient in the 42 kDa apoprotein shows a PS II electron transport activity which is partially sensitive to DCMU [13].

The location of the PS II reaction center on other subunit polypeptides has been suggested purely from a viewpoint of comparative biochemistry. The reaction center of purple photosynthetic bacteria resembles that of PS II in oxygenic photosynthetic organisms in that both have (bacterio)pheophytin and bound quinones as electron acceptors and a site where atrazine specifically binds [14]. The reaction center complex of purple bacteria has a simpler structure consisting of three subunits called L, M and H [14] and the localization of the primary electron donor and acceptor and a bound quinone in the subunits L and M has been demonstrated by X-ray crystallographic studies [15]. Because considerable homologies in amino acid sequence exist between the L and M subunits of purple bacteria and two polypeptides in the 30 kDa region of the PS II reaction center complexes [16,17], and furthermore the sequence homology suggests similar five transmembrane helical spans for the four proteins [18], it has been proposed that the reaction center core of PS II consists of the two 30 kDa polypeptides [15,18–20]. At present, there is no experimental evidence supporting this hypothesis, but most of the PS II preparations in which photochemical activities have been demonstrated are associated with the two 30 kDa polypeptides. A spinach chlorophyll-protein complex containing only the 47 kDa apoprotein has been reported to exhibit the pheophytin photoresponse but the difference spectrum presented is considerably different from that for the pheophytin photoreduction [7].

Here we report the isolation of a PS II preparation from *Synechococcus* which is largely depleted in the two intrinsic polypeptides of 31 and 28 kDa. The preparation was found to retain a full capacity for Q_A photoreduction, indicating that the two 30 kDa polypeptides are not the site of early photochemical events of PS II.

2. MATERIALS AND METHODS

Oxygen-evolving PS II preparations were obtained as reported in [21] from β-octylglucoside-solubilized thylakoid membranes of the thermophilic cyanobacterium, *Synechococcus* sp. grown at 55°C [22]. The preparations were partially purified by DEAE-Toyopearl column chromatography in the presence of 0.1% digitonin [23]. The oxygen-evolving PS II particles (100 μg chl/ml) were incubated with 1% β-octylglucoside in the presence of 0.1 M sucrose, 50 mM Hepes (pH 7.5), 10 mM NaCl and 5 mM $MgCl_2$ at 25°C for 30 min. The suspension was centrifuged at 300000 × *g* for 90 min and the precipitates were suspended in a solution containing 1.0 M sucrose, 50 mM Hepes (pH 7.5), 10 mM NaCl and 5 mM $MgCl_2$. Essentially no chlorophyll *a* was solubilized by the treatment.

Polypeptide compositions were examined by SDS-polyacrylamide gel electrophoresis according to Laemmli [24]. Samples were incubated with 2.5% SDS, 5% 2-mercaptoethanol and 8 M urea for 30 min at 25°C, then run on 10–15% acrylamide gradient slab gels containing 0.1% SDS and 6 M urea. The gels were stained with Coomassie brilliant blue for proteins. Relative quantities of polypeptides were determined by measuring peak areas of bands resolved in the densitogram. Flash-induced absorbance changes of Q_A were determined as reported in [25].

3. RESULTS

The first lane of fig.1 shows polypeptide composition of the partially purified oxygen-evolving particles employed here. The preparation still contained many contaminating proteins as compared with the highly purified oxygen-evolving complexes [23]. In the previous work in which 12.5% acrylamide gels were used, a single polypeptide band of 30 kDa was resolved from the highly purified complexes [23]. The polypeptide band was well resolved into two bands of 31 and 28 kDa in 10–15% gradient gel used in this study. Thus the five subunits of the PS II reaction center complex, i.e., two chlorophyll-carrying subunits of 47 and 40 kDa, the 31 kDa subunit of unknown function, the 28 kDa subunit which has recently been identified as the herbicide-binding protein [5] and the 9 kDa polypeptide which is considered to be the apoprotein of cytochrome *b*-559 are clearly seen.

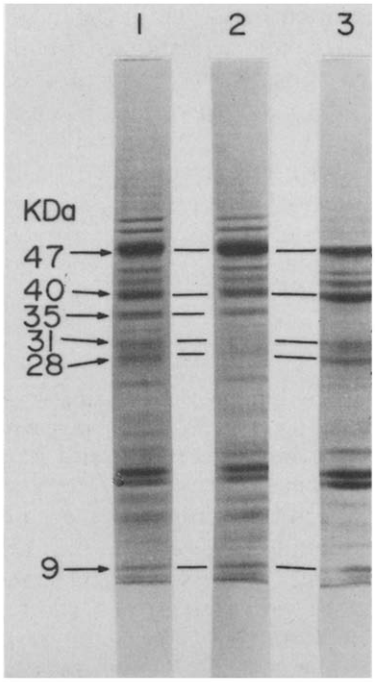

Fig.1. Effect of β-octylglucoside treatment on polypeptide compositions of partially purified oxygen-evolving PS II preparations. Lanes: 1, untreated; 2, β-octylglucoside-treated; 3, PS II reaction center complexes.

In addition, the preparation contained the 35 kDa extrinsic protein. This protein corresponds to the 33–34 kDa protein of chloroplasts that plays an important role in the water oxidation [26]. Considerable amounts of α- and β-subunits (14–16 kDa) of allophycocyanin were still associated with the preparations.

Treatments of the partially purified particles with 1% β-octylglucoside resulted in a selective extraction of the two polypeptides in the 30 kDa region (lane 2). The amounts of polypeptides extracted were estimated by measuring peak area of each polypeptide band relative to that of the 47 kDa band before and after the β-octylglucoside treatment. The detergent extracted about 90% of the 31 kDa polypeptide and 55% of the 28 kDa polypeptide originally present in the particles, whereas the band intensity of the 40 kDa polypeptide was reduced by only 25%, and the 9 kDa polypeptide band was not at all affected. Remarkably, more than 80% of the 35 kDa extrinsic protein was still associated with the particles after the treatment. This indicates that the 28 and 31 kDa polypeptides are not involved in the binding of the 35 kDa protein to the PS II reaction center complex.

As stated in section 1, the two polypeptides of the 30 kDa class are postulated to form the reaction center core containing the primary electron donor and acceptor pair and the bound quinone acceptor [15,18–20]. If this were the case, the removal of the two polypeptides should be accompanied by inactivation of PS II photochemistry. Thus the β-octylglucoside-extracted particles provide a unique opportunity to test this hypothesis. We have determined Q_A photoreduction in the particles before and after the detergent treatment. In partially purified oxygen-evolving particles, flash excitation induced a rapid absorbance increase at 410 nm which returned biphasically to the original level (fig.2, trace a). Kinetics of flash-induced absorbance changes in this wavelength region have been studied in detail with oxygen-evolving preparations and purified PS II reaction center complexes [25]. The initial rapid rise indicates reduction of Q_A, which is present at a ratio of one to 50 chlorophyll *a*, whereas its DCMU-sensitive decay with an overall half-time of several milliseconds represents oxidation of Q_A by Q_B. In the purified PS II reaction center complexes which largely lack Q_B, Q_A is slowly oxidized by ferricyanide added or by a back reaction. Trace a shows that only about a half of Q_A was oxidized with the millisecond kinetics, suggesting that a considerable amount of Q_B had been solubilized during column chromatography in the presence of digitonin. The difference spectrum with three maxima at 415, 440 and 540 nm and troughs at 430 and 550 nm indicates that flash-induced absorption changes are primarily due to Q_A reduction [25,27,28] (fig.3A).

Photoresponses of β-octylglucoside-treated particles were compared with those of untreated particles at the same chlorophyll concentration. Note that the β-octylglucoside treatment had no significant effect on the magnitude of the 410 nm photoresponse, although the millisecond oxidation phase of Q_A was further diminished by the treatment, which partially solubilized the 28 kDa Q_B-protein (fig.2, trace b). Fig.3 shows that the difference spectrum for the flash-induced absorbance changes in the β-octylglucoside-treated prepara-

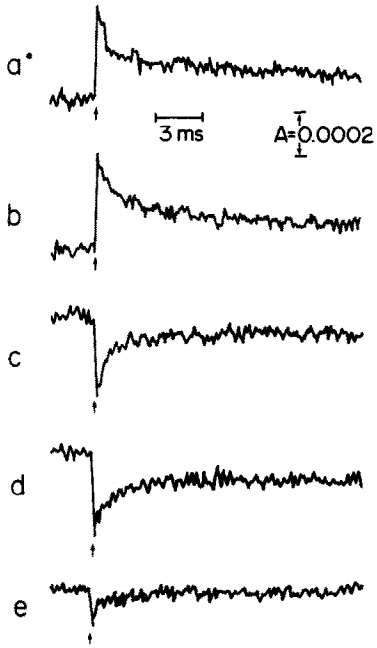

Fig.2. Effect of β-octylglucoside treatment on flash-induced absorbance changes at 410 and 430 nm. Traces: a, untreated, 410 nm; b, β-octylglucoside-treated, 410 nm; c, untreated, 430 nm; d, β-octylglucoside-treated, 430 nm; e, d + 100 μM benzidine. The reaction mixture contained 1 M sucrose, 50 mM Hepes (pH 7.5), 10 mM NaCl, 5 mM $MgCl_2$, 0.2 mM ferricyanide and untreated or β-octylglucoside-treated PS II preparations equivalent to 4.3 μg chl/ml. Saturating laser flashes with a half-peak height duration of 300 ns passed through two Toshiba VR-65 filters. The flash repetition rate was 1 Hz and 40 signals were averaged.

tion (B) was essentially the same as that of untreated sample (A) except that an absorption change occurred between 450 and 480 nm in the detergent-treated preparation (carotenoid?). Because the difference spectra of untreated and β-octylglucoside-treated preparations were determined at the same chlorophyll concentration, and because solubilization of chlorophyll *a* was negligible during the detergent treatment, it is concluded that the β-octylglucoside-treated preparation still retains a full capacity for the Q_A photoreduction. Thus the 31 and 28 kDa polypeptides are not needed for Q_A photoreduction to occur. It follows that P680 and pheophytin are not located on the two polypeptides.

Fig.2 suggests that the β-octylglucoside treatment affects electron transport on the water side of

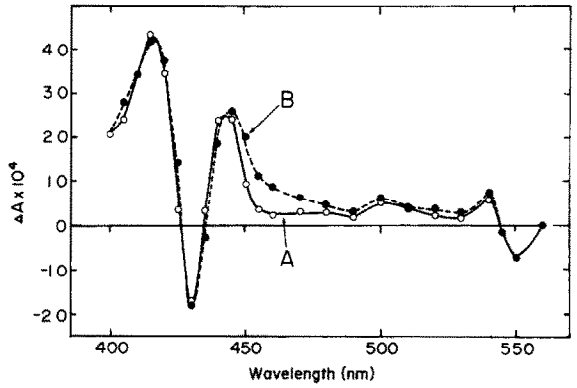

Fig.3. Difference spectra of flash-induced absorbance changes in oxygen-evolving PS II preparations. (A) Untreated, (B) β-octylglucoside-treated. The spectra were determined in the presence of 0.1 mM benzidine to isolate the Q_A photoresponse (see text). Chlorophyll concentration was 3.1 μg chl/ml both in A and B. Other experimental conditions were as in fig.2.

the reaction center. Recovery of the flash-induced absorbance increase at 430 nm was markedly slowed down by the treatment (cf. traces c and d). This indicates an inhibition of electron flow from water to P680 (or Z) because the slow recovery was mostly eliminated on addition of benzidine (trace e) which rapidly donates electrons to Z (or P680) [25]. Thus, the results suggest that the 31 kDa polypeptide plays some role in electron flow on the water side of the PS II reaction center. The difference spectra shown in fig.3 were determined in the presence of benzidine to eliminate the 430 nm photoresponse which may be ascribed to $P680^+$, Z^+ or a related electrochromic shift [28].

4. DISCUSSION

The present work demonstrates that a *Synechococcus* oxygen-evolving preparation which is largely depleted of the two intrinsic polypeptides of the 30 kDa class has a full capacity for photoreducing Q_A. Evidently, the two polypeptides are not essential for electron transport from P680 to Q_A. The results strongly argue against the view that P680, pheophytin and Q_A are located on the two 30 kDa polypeptides.

Synechococcus PS II reaction center complex contains, besides the two 30 kDa polypeptides, three subunits of 47, 40 and 9 kDa [12]. Chlorophyll *a* is associated with the 47 and 40 kDa

subunits but not with the 9 kDa polypeptide which is considered to be the apoprotein of cytochrome *b*-559. The chlorophyll-carrying 40 kDa polypeptide is not the site of primary photochemistry of PS II because a PS II reaction center complex which contains the 47 kDa apoprotein together with reduced amounts of small subunits but no 40 kDa polypeptide exhibits photoreduction of Q_A and pheophytin [12]. Taking the results obtained here together with the previous observations, it is concluded that P680, pheophytin and Q_A are located on the 47 kDa chlorophyll-carrying polypeptide. This conclusion is supported by photoaffinity labelling experiments which show that the 47 kDa polypeptide has a plastoquinone-binding site [5]. The location of Q_A on the 47 kDa polypeptide has previously been suggested from the observation that the amount of Q_A remaining in the PS II complex lacking the 40 kDa subunit is considerably larger than that of the two 30 kDa polypeptides [12].

In view of the similar subunit structure of the PS II reaction center complex between *Synechococcus* [11] and spinach [2], the 31 kDa polypeptide would correspond to the D-2 protein [17,29] in chloroplasts, the function of which is in dispute. The high sequence homology between the Q_B-protein and the D-2 protein suggests that the D-2 protein has a plastoquinone-binding site [17]. The present work indicates that the 31 kDa polypeptide is not the Q_A-binding protein but may play some role in electron transport on the water side of PS II. A possibility remains that the polypeptide carries Z which is assumed to be a bound plastoquinone [30]. At any event, the β-octylglucoside-treated oxygen-evolving preparation would serve as a very interesting and powerful tool for the investigation of the role of the 31 kDa polypeptide in the PS II complex. Experiments along this line are in progress.

Finally, the present work also provides important information on the subunit organization of the oxygen-evolving complex. The β-octylglucoside treatment which solubilized most of the 31 kDa polypeptide and nearly half of the 28 kDa polypeptide left more than 80% of the 35 kDa polypeptide bound to the preparation. This indicates that the two intrinsic polypeptides are not essential for the binding of the 35 kDa polypeptide to the PS II reaction center complex. We suggest, therefore, that the 35 kDa polypeptide binds to one or both of the two chlorophyll-carrying polypeptides of the reaction center complex.

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