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# Immunological studies on the D-1 and D-2 proteins of photosystem II preparations from the thermophilic cyanobacterium, *Synechococcus* sp.

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Immunoblotting of *Synechococcus* photosystem II preparations with site-directed antisera against the spinach D-2 protein and an antiserum against a large polypeptide segment of the spinach D-1 protein showed (i) that the 28 and 31 kDa polypeptides of the cyanobacterial photosystem II core complex are equivalent to the D-1 and D-2 proteins of higher plants and algae and (ii) that the β-octylglucoside treatment of the photosystem II preparation diminishes band intensities of the 28 and 31 kDa proteins in Coomassie blue-stained gels mainly by increasing the amounts of two proteins which migrate as a dimer of $D_1$ or $D_2$ or its aggregate, and of proteolytic digestion products.

D-1 protein; D-2 protein; Photosystem II; Antibody; (*Synechococcus*)

## 1. INTRODUCTION

The two essential polypeptide subunits of PS II core complexes in the 30 kDa molecular mass region are called the D-1 and D-2 proteins [1]. The D-1 protein is a herbicide- and $Q_B$-binding protein [1–3], while the function of the D-2 protein until recently was less clear. Sequence homologies of the D-1 and D-2 proteins with those of the L and M subunits of photosynthetic bacterial reaction centers [4,5] lead to a proposal that the D-1 and D-2 proteins constitute the reaction center of PS II [6,7]. Recently, a complex, which consists of the D-1 and D-2 proteins and cytochrome *b*-559 and exhibits the primary photochemistry of PS II, has been isolated from spinach [8] and pea [9].

A purified PS II core complex isolated from the thermophilic cyanobacterium, *Synechococcus* sp., consists of 47, 40, 31, 28 and 10 kDa subunit polypeptides [10]. The 47 and 40 kDa polypeptides carry chlorophyll *a* and β-carotene, and the 10 kDa polypeptide is an apoprotein of cytochrome *b*-559. The 28 kDa polypeptide was identified as the D-1 protein by photoaffinity labeling with azido-derivatives of atrazine, monuron and dinoseb [11].

In this communication, we report cross-reactivity of site-directed antisera against the spinach D-2 protein [12] and of an antiserum against the spinach D-1 protein [13] with the subunit polypeptides in the 30 kDa region of *Synechococcus* PS II preparations. The 31 kDa polypeptide was identified as the D-2 protein of the cyanobacterium. Immunoblotting with the antisera has also permitted detection of very small amounts of the 28 and 31 kDa polypeptides in a 40 kDa polypeptide preparation. In addition, aggregates and proteolytic digestion products of the two polypeptides could be detected, which had not been visible in CBB-stained gels. This leads to an altered interpretation of the role of the 47 kDa polypeptide as discussed in previous work [10,14].

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*Abbreviations:* PS, photosystem; CBB, Coomassie brilliant blue

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## 2. MATERIALS AND METHODS

Oxygen-evolving PS II preparations were prepared from the thylakoid membranes of the thermophilic cyanobacterium, *Synechococcus* sp., as in [15] and were partially purified by passage through a Sepharose CL-4B column after incubation with 0.5% deoxycholate for 30 min [16]. PS II core complexes, the complexes which lack the 40 kDa polypeptide (CP2-b) and the 40 kDa chlorophyll-protein complexes (CP2-c) were prepared as in [10].

SDS-polyacrylamide gel electrophoresis was carried out as reported previously [11]. Three sets of samples were applied to 11.5–15% acrylamide gels and, after electrophoresis, one set was stained with CBB, while the rest were transferred to nitrocellulose membranes for immunoblotting [17]. Antisera against synthetic oligopeptides corresponding to the amino acid sequences from $Ala_{230}$ to $Ala_{235}$ and from $Ala_{235}$ to $Ala_{241}$ of the D-2 protein were prepared by Geiger et al. [12]. An antibody against the D-1 protein was prepared against a fusion protein consisting of a part of the D-1 protein (amino acids 167–353) and galactosidase which had been expressed in *E. coli* [13].

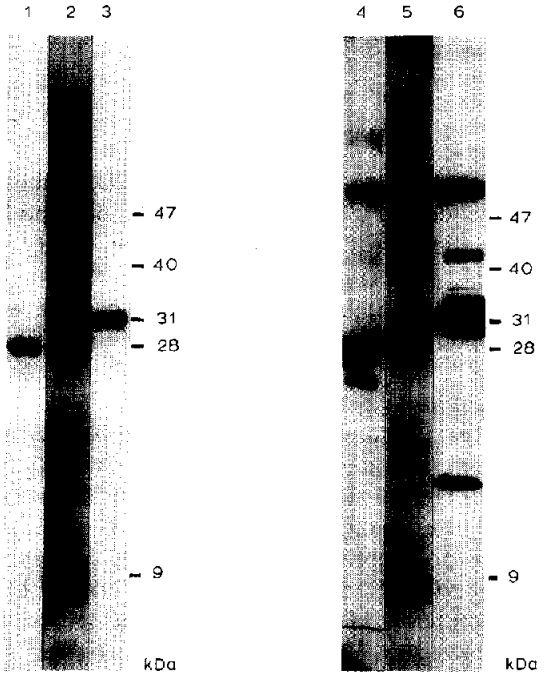


Fig.1. Cross-reactivity of the antisera against the spinach D-1 and D-2 proteins with constituent polypeptides of *Synechococcus* PS II core complexes. Lanes: 2 and 5, CBB-stained polypeptide patterns of the PS II core complexes; 1 and 4, immunoblots of an antiserum against spinach D-1 protein (amino acids 167–353); 3 and 6, an antiserum against spinach D-2 protein (amino acids 235–241). Amounts of PS II core complexes loaded correspond to 1.5 $\mu$g (lanes 1 and 3) and 12 $\mu$g chlorophyll *a* (lanes 2,4–6).

## 3. RESULTS

Cross-reactivity of the site-directed antisera, which had been raised against sequences of the spinach D-1 and D-2 proteins, with the *Synechococcus* 28 and 31 kDa polypeptides was examined by immunoblotting (fig.1). The 28 kDa polypeptide has already been identified as the D-1 protein ($Q_B$- and herbicide-binding protein) by photoaffinity labeling experiments [11]. Fig.1 confirms that the 28 kDa polypeptide indeed is the D-1 protein. The antiserum against a polypeptide segment ($AA_{167}$–$AA_{353}$) of the spinach D-1 protein reacted with the 28 kDa polypeptide (lane 1). Two site-directed antisera against the amino acid sequences from $AA_{235}$ to $AA_{241}$ (lane 3) and from $AA_{230}$ to $AA_{235}$ (not shown) of spinach D-2 protein cross-reacted with the 31 kDa polypeptide. Thus, the 31 kDa polypeptide is the D-2 protein of the cyanobacterium.

When large amounts of the PS II core complexes were applied to gels, several other bands,

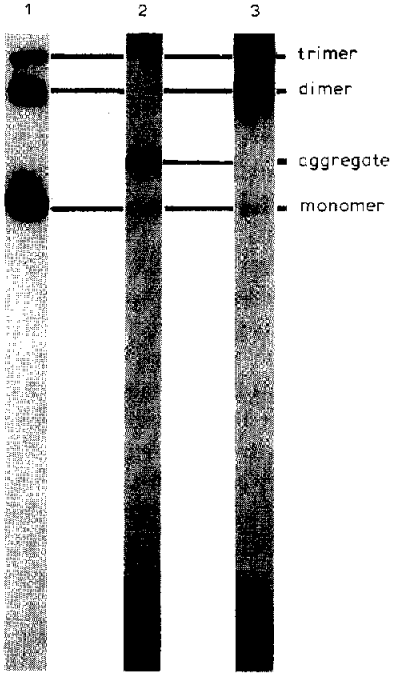


Fig.2. Re-electrophoresis and immunoblot with an antiserum against spinach D-2 protein (amino acids 235–241) of excised D-2 monomer (31 kDa; lane 1), aggregate (between 47 and 40 kDa; lane 2) and homo/heterodimer (above 47 kDa; lane 3).

which reacted with either the D-1 or D-2 antiserum or both, appeared (lanes 4,6). Bands, which migrated faster than the 28 and 31 kDa polypeptides, are ascribed to partial proteolytic degradation products of the respective polypeptides. A dense band which appeared above the 47 kDa polypeptide reacted with both the D-1 and D-2 antisera. A faint band, which migrated between the 47 and 40 kDa polypeptides, also reacted with the two antisera. The relative intensity of the two bands varied considerably in different experiments. The band which migrates above the 47 kDa polypeptide strongly suggests the formation of either homodimers or a heterodimer of the D-1 and D-2 proteins. A differentiation between homodimers or heterodimer cannot be made, because only one single band in this region is observed. This is in contrast to spinach PS II reaction center complexes which yielded $(D\text{-}1)_2$ and $(D\text{-}2)_2$ dimers separately [18]. The faint band positioned between the 47 and 40 kDa polypeptides is larger than D-1 or D-2 alone and may be attributed to an aggregate between D-1 or D-2, respectively, and an as yet unknown polypeptide of about 10–15 kDa.

In order to clarify this situation, D-2 monomer (at 31 kDa), aggregate (between 47 and 40 kDa) and homo/heterodimer (above 47 kDa) were excised from the gel and subjected to re-electrophoresis in a 15% polyacrylamide gel system. The results are presented in fig.2. Upon electrophoresis of the D-2 monomer, the formation of a dimer and even

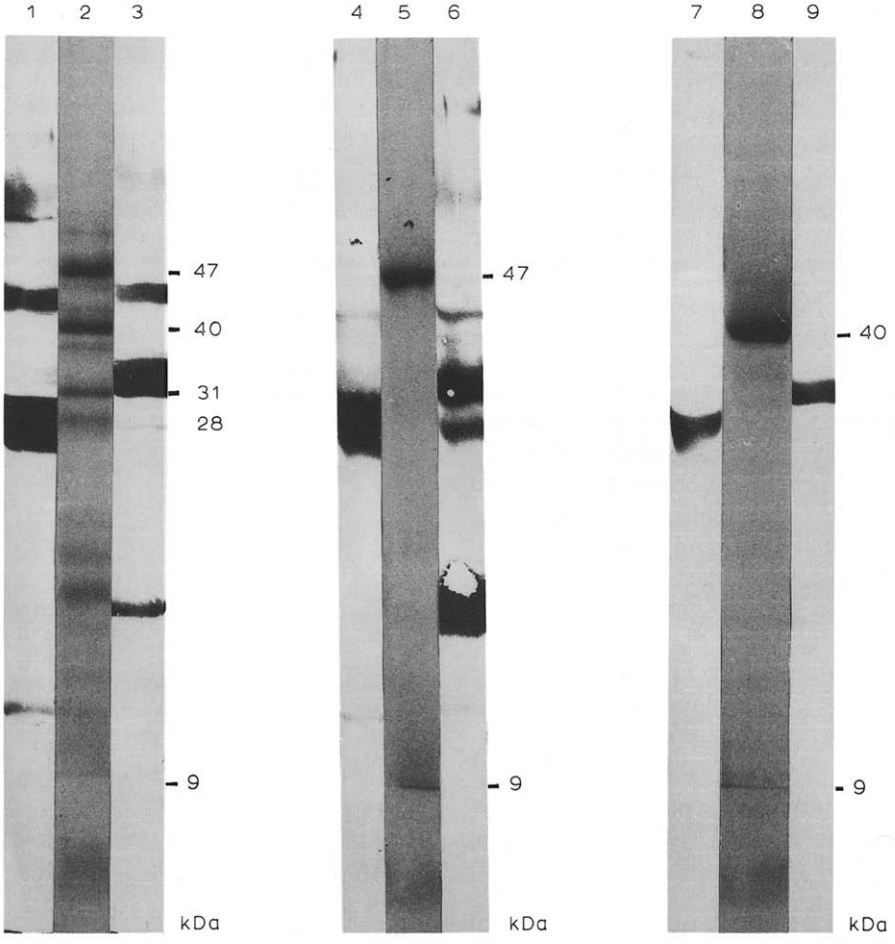


Fig.3. Presence of the D-1 and D-2 proteins in various chlorophyll-protein complexes. Lanes: 1–3, partially purified oxygen-evolving PS II preparation; 4–6, CP2-b; 7–9, CP2-c; 2,5 and 8, CBB-stained gels; 1,4 and 7, immunoblots with the antiserum against spinach D-1 protein (amino acids 167–353); 3,6 and 9, immunoblots with the antiserum against spinach D-2 protein (amino acids 235–241).

a trimer is observed (lane 1). The aggregate obviously partially breaks down to the D-2 monomer (lane 2). The dimer also, to a small extent, breaks down to the D-2 monomer, and in addition, the trimer is formed (lane 3).

The *Synechococcus* PS II preparation is resolved by SDS-polyacrylamide gel electrophoresis into two chlorophyll-protein complexes, CP2-b which is the complex lacking the 40 kDa polypeptide and CP2-c, the isolated chlorophyll-bearing 40 kDa polypeptide [10]. Fig.3 shows Western blots of the three chlorophyll-protein complexes. The partially purified oxygen-evolving PS II preparation showed one or two degradation products of the D-1 and D-2 protein (lanes 1,3), respectively. The homodimer or the heterodimer of the two proteins was less abundant than the aggregate between D-1 and D-2 protein, respectively, and the small unknown polypeptide. The CP2-b preparation used here contained only small amounts of the 28 and 31 kDa polypeptides as judged from CBB-stained gels (lane 5). The presence of these two polypeptides was, however, clearly demonstrated in immunoblots. Note that the proteolytic products of the D-2 protein increased markedly in CP2-b as compared to the untreated PS II preparation, suggesting that the protein becomes more susceptible to endogenous protease after SDS-treatment. Unexpectedly, CP2-c, which has no visible bands of the 28 and 31 kDa polypeptides in CBB-stained gels, showed clear bands of the D-1 and D-2 proteins. Thus, in contrast to the previous conclusion [10], the 40 kDa polypeptide is associated with small but significant amounts of these two proteins.

Previous work has shown that the band intensity of the 28 and 31 kDa polypeptides in CBB-stained gels considerably decreased by washing of the oxygen-evolving PS II preparation with high concentrations of $\beta$-octylglucoside without any significant decrease either in band intensities of other polypeptides or in the magnitude of $Q_A$ photoreduction [14]. It was suggested, therefore, that the 28 and 31 kDa protein do not carry the PS II reaction center [14]. The results shown in figs 1 and 3 in this paper indicate, however, that the CBB-stained bands in the 30 kDa region are not necessarily a good measure of the 28 and 31 kDa polypeptides. Therefore, we have reexamined the detergent-effect using the antisera (fig.4). As shown previously, the CBB-stained bands of the 28 and 31 kDa polypeptides were markedly diminished by the detergent treatment (lane 5). Corresponding bands in the immunoblots also decreased appreciably (lanes 1,3,4 and 6). Note, however, that the detergent-treatment caused a significant increase in the amount of the two proteins which migrate as an aggregate (lanes 4 and 6). In addition, proteolytic digestion of the D-2 protein was markedly enhanced by the detergent-treatment. Thus, the total amounts of the D-1 and D-2 proteins present in the $\beta$-octylglucoside-washed preparation are not so low as the CBB-stained gels suggest.

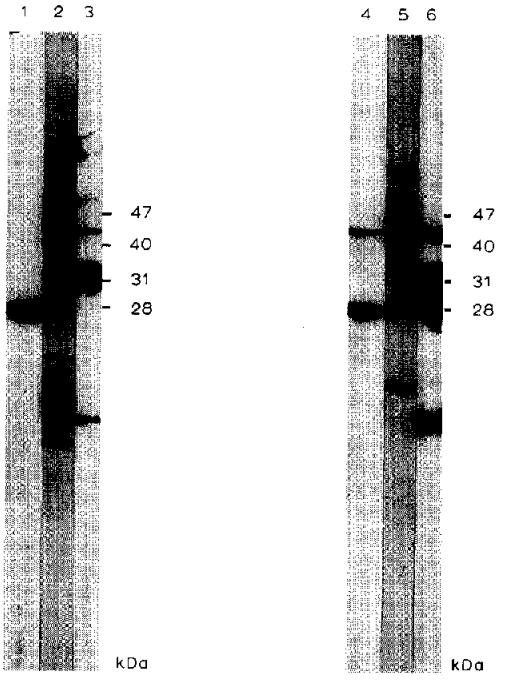


Fig.4. Effects of $\beta$-octylglucoside treatments on polypeptide compositions of *Synechococcus* PS II preparations. Lanes: 2 and 5, CBB-stained polypeptide pattern of a partially purified oxygen-evolving PS II preparation from *Synechococcus* sp. before and after the $\beta$-octylglucoside treatment. The detergent treatment was carried out by incubating the PS II preparation with 2% $\beta$-octylglucoside for 30 min at 25°C. Then, the preparation was precipitated by centrifugation (300000 × *g*, 60 min). Lanes: 1 and 4, immunoblots of the oxygen-evolving PS II preparation with antiserum against spinach D-1 protein (amino acids 167–353) before and after the detergent-treatment; 3 and 6, immunoblots of the oxygen-evolving PS II preparation with antiserum against spinach D-2 protein (amino acids 235–241) before and after the detergent treatment.

## 4. DISCUSSION

The present work confirms that the 28 kDa polypeptide of *Synechococcus* PS II core complexes is the D-1 protein and further indicates that the 31 kDa polypeptide is the D-2 protein of the cyanobacterium. Immunological identification of the two polypeptides of the 30 kDa molecular mass class in wheat [19] and spinach [12,18] as the D-1 and D-2 proteins has been reported. Thus, the results obtained here provide additional evidence that the molecular organization of the PS II core complexes is similar between higher plants and the photosynthetic procaryote. Furthermore, cross-reactivity of the antisera against the oligopeptides corresponding to $AA_{230}$-$AA_{235}$ and $AA_{235}$-$AA_{241}$ of the spinach D-2 protein with the 31 kDa polypeptide of *Synechococcus* suggests that high homology exists between the spinach and *Synechococcus* D-2 proteins in this area. In particular, the sequence from $AA_{230}$ to $AA_{241}$ is situated in a domain of the protein which is proposed to involve a plastoquinone binding niche [20]. This region of the sequence has been well conserved during evolution of the photosynthetic apparatus from cyanobacteria to higher plants.

The present work provides important information on the molecular organization of the PS II core complex. It has been reported that the D-1 and D-2 proteins, together with cytochrome *b*-559, constitute a central part of the PS II core complex [8,9]. Homodimers or a heterodimer of the D-1 and D-2 proteins were detected from the PS II preparations by SDS gel electrophoresis. Isolation of CP2-b indicates that the heterodimer directly attaches to the 47 kDa polypeptide [10]. The results obtained here further show that the 40 kDa polypeptide preparation is associated with a small amount of the two proteins. As in higher plants, the 40 and 47 kDa polypeptides are in the position to collect excitation energy from the light-harvesting pigment-protein of PS II and transfer it to the central heterodimer.

It has been suggested that the PS II reaction center is not associated with the 28 and 31 kDa protein because the two protein bands resolved by SDS gel electrophoresis were preferentially diminished by $\beta$-octylglucoside treatment of *Synechococcus* oxygen-evolving PS II preparations without a significant loss of $Q_A$ photoreduction [14]. The results obtained here do not support this assumption anymore. The diminished intensities of the 28 and 31 kDa bands in CBB-stained gels are mostly ascribed to migration of the two proteins as aggregates, or proteolytic digestion products, rather than detachment of the two proteins from the core complex. Apparently, $\beta$-octylglucoside has two different effects: (i) a stabilization of the association of the D-1 and D-2 proteins against SDS, which results in an increase in the amounts of the heterodimer, and (ii) an activation of an endogenous protease, which preferentially digests the D-2 protein. Thus, the present work cautions that CBB-stained bands in the 30 kDa region are not necessarily good measures of the D-1 and D-2 proteins in detergent-treated PS II preparations.

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