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Protein composition of the photosystem II core complex in genetically engineered mutants of the cyanobacterium *Synechocystis* sp. PCC 6803

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Abstract. The presence of four photosystem II proteins, CP47, CP43, D1 and D2, was monitored in mutants of *Synechocystis* sp. PCC 6803 that have modified or inactivated genes for CP47, CP43, or D2. It was observed that: (1) thylakoids from mutants without a functional gene encoding CP47 are also depleted in D1 and D2; (2) inactivation of the gene for CP43 leads to decreased but significant levels of CP47, D1 and D2; (3) deletion of part of both genes encoding D2, together with deletion of part of the CP43-encoding gene causes a complete loss of CP47 and D1; (4) thylakoids from a site-directed mutant in which the His-214 residue of D2 has been replaced by asparagine do not contain detectable photosystem II core proteins. However, in another site-directed mutant, in which His-197 has been replaced by tyrosine, some CP47 as well as breakdown products of CP43, but no D1 and D2, can be detected. These data could indicate a central function of CP47 and D2 in stable assembly of the photosystem II complex. CP43, however, is somewhat less critical for formation of the core complex, although CP43 is required for a physiologically functional photosystem II unit. A possible model for the assembly of the photosystem II core complex is proposed.

Introduction

Light-induced plastoquinone reduction by water, a key process in photosynthesis, depends on functional integrity of the photosystem II (PS II) complex. This complex consists of a "core" of at least five integral membrane proteins: the chlorophyll-binding proteins CP47 and CP43, the 32–34 kDa proteins D1 and D2, and cytochrome b_{559} (Satoh 1985; Arntzen and Pakrasi 1986). In addition, one to several extrinsic proteins also participate in PS II function. The precise role of the individual proteins of the complex is still the subject of speculation. Moreover, it is as yet largely unknown how the

assembly of the PS II core complex depends on the presence of each individual polypeptide of that complex.

A rather fruitful approach to study the assembly processes in PS II has been to isolate and characterize mutants with altered PS II properties. Although characterization of mutants that were obtained by random mutagenesis or spontaneous mutations have yielded some important insight into the requirements for PS II core complex function or assembly (Miles et al. 1979, Bishop 1982, Metz et al. 1986, Jensen et al. 1986), it is often difficult to distinguish whether an observed change in one of the proteins should be attributed to modification of its structural gene, or to the pleiotropic effects of a mutation in another gene.

Recent advances in molecular genetics have allowed the construction of cyanobacterial mutants in which the gene encoding a component of the PS II complex has been specifically inactivated or modified (Williams 1987, Golden et al. 1987, Sherman et al. 1984). Using the photoheterotrophic cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis* 6803), which can be propagated on glucose-containing medium in the light in the absence of PS II activity, mutants with inactivated genes for CP47, cyt b_{559}, D2 and/or CP43 have been developed by specific interruption of these genes (Williams 1987, Vermaas et al. 1987a, Pakrasi et al. 1987, J.G.K. Williams, W.F.J. Vermaas and C.J. Arntzen, unpublished results). Moreover, site-directed mutations have been introduced to replace crucial histidine residues of the D2 protein (Vermaas et al. 1987b).

In many *Synechocystis* 6803 mutants with modified PS II, the overall PS II function (light-driven electron transport from water to plastoquinone) was inactivated completely by the gene modifications. In various mutants, CP47 and/or CP43 were still present (Vermaas et al. 1986, Vermaas et al. 1987c, Pakrasi et al. 1987), albeit in apparently decreased quantities in some of these mutants. However, since there are many proteins in the thylakoids from *Synechocystis* 6803, and since we have not yet succeeded in obtaining a pure PS II preparation from this organism, we could not observe whether the two 32–34 kDa proteins were still present in these mutants. At the time of those experiments no suitable antibodies against D1 or D2 were available to us. An unambiguous interpretation of the impaired PS II activity of the different mutants was not possible because of the uncertainty concerning the presence of the D1 and D2 proteins.

To address this problem, we have raised polyclonal antibodies against the spinach D1 and D2 proteins, and these antibodies show good cross-reactivity with *Synechocystis* 6803 D1 and D2. The results of immunoreactions of antibodies against D1, D2, CP43 and CP47 with thylakoid proteins from the various directed PS II mutants are reported in this paper. The data suggest

that both CP47 and D2 appear to be required for stable integration and assembly of the PS II core complex.

Materials and methods

Synechocystis sp. PCC (Pasteur Culture Collection) 6803 was cultivated in BG-11 medium as described previously (Vermaas et al. 1987a). Mutant cells of this organism were grown in BG-11 supplemented with 5 mM glucose and the appropriate antibiotic(s) against which a mutant was resistant. The final concentration of antibiotic was 20, 30 and 7 μg/ml for kanamycin, spectinomycin and chloramphenicol, respectively. For obligate photoheterotrophic growth, a PS II electron transport inhibitor (25 μM atrazine) was added.

For preparation of thylakoids, cells were harvested and resuspended in 25 mM HEPES (pH = 7.5), 0.3 M sorbitol, 25 mM NaCl, 1 mM phenylmethylsulfonylfluoride, 1 mM ε-aminocaproic acid and 1 mM benzamidine (resuspension medium) to a concentration of 1 mg chlorophyll per ml. The cells were passed twice through a French Press at 1500 kg/cm^2. Unbroken cells were removed by centrifugation at 5000 $\times$ g for 10 min. The thylakoid fraction was collected by centrifugation at 37,000 rpm in a RP50-2 rotor (Hitachi) for 1 hour, and resuspended in the resuspension medium.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of thylakoid proteins was performed essentially according to the method described by Chua (1980); however, both stacking and resolving gel contained 5.5 M urea, and 12.5% acrylamide was used for the resolving gel. Thylakoids were solubilized in a buffer containing 60 mM Tris/HCl (pH 8.0), 2% lithium dodecyl sulfate, 60 mM dithiothreitol and 20% (w/v) sucrose. After running the gels, thylakoid proteins were blotted (30 V; 16 hrs) onto nitrocellulose (BioRad) in 25 mM Tris, 192 mM glycine, 20% (v/v) methanol and 0.01% (w/v) sodium dodecyl sulfate. Coomassie Brilliant Blue staining of the blotted gel and Amido Black staining of the blot showed that this procedure yielded an effective protein transfer to the nitrocellulose.

To raise antibodies, CP43 and CP47 were prepared by isoelectric focusing of PS II membrane proteins in the presence of β-octylglucopyranoside. Spinach PS II membrane particles (Berthold et al. 1981) were treated with 0.8 M Tris/HCl (pH 8.5) and solubilized with 60 mM β-octylglucopyranoside in 10% (w/v) sucrose, 20 mM Tricine/NaOH (pH 8.5), 10 mM NaCl and 0.1% (w/v) Ampholine (pH 3–10, LKB). The sample was layered in the middle of a linear gradient of 0.05–0.2% (w/v) Ampholine (pH 4-6.5) and

0-20% (w/v) sucrose containing 60 mM β-octylglucopyranoside and electrofocused at 300 V for 12 hrs at 0° C. A green band at pI 5.4 was collected and centrifuged at 224,000 × *g* for 1 hr. Both CP43 and CP47 remained in the supernatant. This supernatant was diluted with 20 mM MES/NaOH (pH 6.5) and 10 mM NaCl to make a final concentration of 35 mM β-octylglucopyranoside, layered on a 30% (w/v) sucrose cushion containing 35 mM β-octylglucopyranoside, 20 mM MES/NaOH (pH 6.5) and 10 mM NaCl, and centrifuged at 224,000 × *g* for 5 hrs. CP47 and CP43 were recovered in the green precipitate and in the green band in the supernatant, respectively. D1 and D2 were prepared from PS II reaction center complexes (Ikeuchi et al., 1985) by gel electrophoresis. Protein bands were stained with 1% (w/v) Coomassie Brilliant Blue R-250 in water for 10 min, D1 and D2 bands were cut out, and then again subjected to gel electrophoresis. D1 and D2 were eluted from the gel strips by electrophoresis (Mendel-Hartvig 1982), dialyzed against water, and lyophilized. 50-100 μg of each protein was injected into a rabbit according to Chua et al. (1982).

The nitrocellulose thylakoid protein blots were probed with one of these polyclonal antibodies, which was detected using an assay kit (BioRad, Richmond CA) employing horse radish peroxidase (for D1 and D2 antibodies) or alkaline phosphatase (for CP43 and CP47 antibodies) conjugated to antibodies (from goat) against the rabbit antibody F_c portion, following the procedures recommended by the manufacturer of the assay kit.

Results

Mutant characteristics

Some properties of the mutants that have been used in this study have been summarized in Table 1. The genetic characteristics of some mutants (*psbB*-I1, *psbDC*-D1, *psbD*-S197Y and *psbD*-S214N) have been published (Vermaas et al. 1987a,b). The name of each mutant is composed of the targeted gene(s) for mutagenesis, followed by an abbreviation of the type of mutation made (Insertion, Deletion, Chimaeric construction, or Site-directed mutation), and a number. For site-directed mutations, the number represents the amino acid residue that has been mutated, and is followed by the one-letter code of the residue into which it has been changed. The nature of the genetic changes in the others has been summarized below.

The first five mutants listed in Table 1 were generated by transformation of wild-type *Synechocystis* 6803 with a construction containing the spinach *psbB* gene (coding for CP47) flanked on both sides by the flanking regions

Table 1. Site(s) of genetic modification in the *Synechocystis* 6803 mutants.

mutant	gel lane (Fig. 2–5)	modified gene	nature of modification	PS II ET	CP47	CP43	D2	D1
psbB-C1	2	*psbB*	part of gene replaced by *psbB*	+	++	++	++	++
psbB-C2	3	*psbB*	from spinach. It is not yet known	+	++	++	++	++
psbB-C3	4	*psbB*	how far the spinach sequence ex-	−	−	++	+	+
psbB-C4	5	*psbB*	tends in each case. *neo* insertion	−	−	++	+	+
psbB-C5	6	*psbB*	just beyond the 3′ end of *psbB*	+	++		++	++
psbB-I1	7	*psbB*	*neo* insertion in *psbB* 182 bp before 3′ end	−	−	+	−	−
psbC-I1	8	*psbC*	*neo* insertion in *psbC* 154 bp before the 3′ end	−	+	−	+	+
psbC-I2	9	beyond *psbC*	*neo* insertion 172 bp beyond the 3′ end of *psbC*	+	++	++	++	++
psbDC-D1	10	*psbD1* *psbD2* *psbC*	part of *psbD1* and *psbC* deleted and replaced by DNA fragment leading to Cm^R. Part of *psbD2* deleted and replaced by DNA fragment leading to Sm^R	−	−	−	−	−
psbD-S197Y	11	*psbD1* *psbD2*	single-base change in *psbD1*; leads to replacement of His-197 by Tyr in D2; part of *psbD2* replaced by Sm^R. *neo* inserted 172 bp beyond *psbC* 3′	−	+	*	−	−
psbD-S214N	12	*psbD1* *psbD2*	as *psbD*-S197Y, but containing a single-base change leading to replacement of His-214 by Asn in D2, and containing the wild-type His-197 in D2	−	−	−	−	−

CP47 and CP43 are encoded by *psbB* and *psbC*, respectively. The two copies of *psbD*, *psbD1* and *psbD2*, both code for the D2 protein. PS II ET: electron transport from water to plastoquinone. The absence of electron transport is indicated by "−", its presence by "+". Apparently normal levels of any of the four proteins is indicated by "++", decreased levels by "+", and apparent absence of any of the proteins from the thylakoid by "−". *: breakdown product detectable. Cm^R: chloramphenicol resistance; Sm^R: spectinomycin resistance; *neo*: DNA fragment containing the neomycin phosphotransferase gene leading to kanamycin resistance.

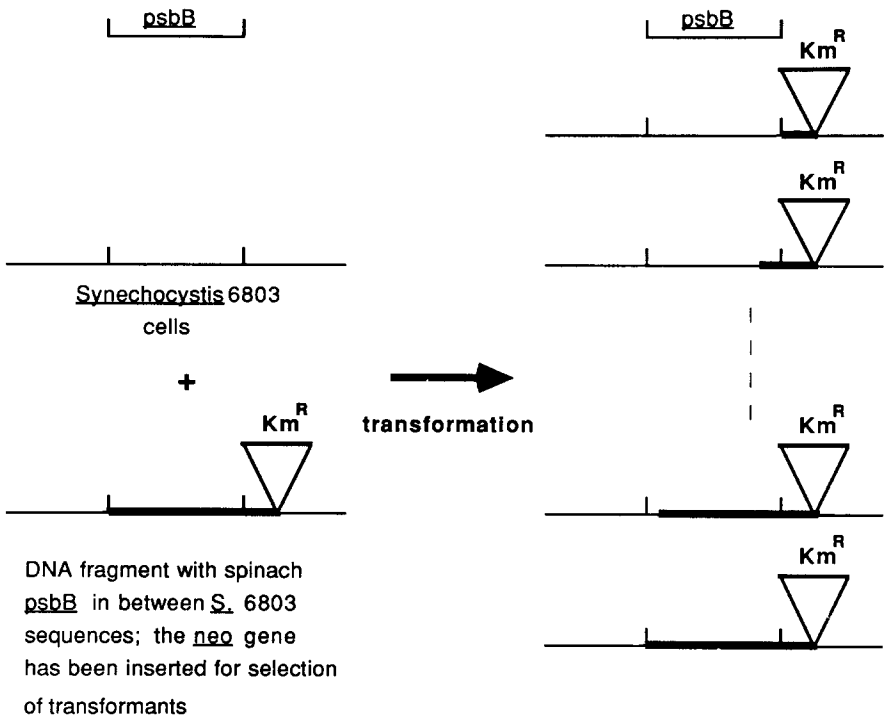

Fig. 1. Scheme for construction of cyanobacterial transformants that contain a chimeric spinach/*Synechocystis* 6803 *psbB* gene. Wild-type *Synechocystis* 6803 cells were transformed with a DNA fragment that contained: (1) a 0.2 kb *HpaI*/*NcoI* fragment upstream of *psbB* obtained from *Synechocystis* 6803 (——); (2) an *NcoI*/*DraI* fragment from spinach containing spinach *psbB* and a short (0.1 kb) downstream region (▬); (3) a 0.4 kb *DraI*/*KpnI* *Synechocystis* 6803 fragment from downstream of *psbB* (——); and (4) a DNA fragment containing a neomycin phosphotransferase gene (——) leading to kanamycin resistance (Km^R) between (2) and (3). The *NcoI* site in (1) had been induced by site-directed mutagenesis of a single base just outside the *psbB* coding region in order to allow an in-phase linkage of (1) and (2) at the *psbB* start site. For a precise location of the restriction sites, see the sequences as published by Vermaas et al. (1987a) and Morris and Herrmann (1984). After transformation, kanamycin-resistant transformants were selected. To obtain such transformants, cross over had to take place (1) in the homologous region downstream of *psbB* and the DNA fragment containing the neomycin phosphotransferase gene, and (2) in a region of homology between spinach and cyanobacterial *psbB*, or in the homologous region upstream of *psbB*. Various possible transformants have been indicated in the Figure. In principle a large number of different transformants is expected to be created, depending on where the cross over in *psbB* occurred.

of *psbB* from *Synechocystis* 6803 (see Fig. 1). For convenient selection of transformants, a DNA fragment containing the neomycin phosphotransferase gene (the *neo* gene) had been inserted at the spinach/*Synechocystis* 6803 junction downstream of *psbB*. Integration of spinach *psbB* into the

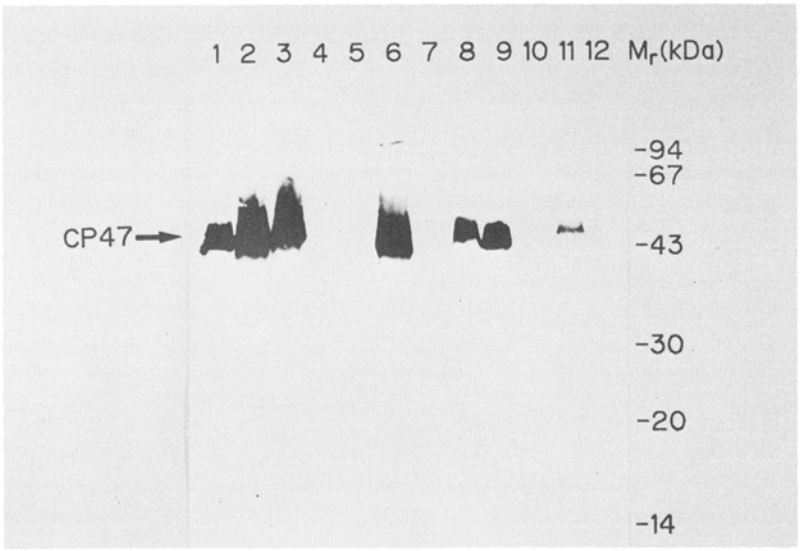

Fig. 2. Western blot of thylakoid proteins from wild type and mutant *Synechocystis* 6803 probed with rabbit polyclonal antibodies raised against the spinach CP47 protein. Per lane, 3 μg chlorophyll was loaded. The location of the molecular mass markers is indicated. Lane 1: wild type; lane 2-6: spinach/cyanobacterial *psbB* hybrids *psbB*-C1, *psbB*-C2, *psbB*-C3, *psbB*-C4, and *psbB*-C5, respectively; lane 7: *psbB*-I1 (interrupted *psbB*); lane 8: *psbC*-I1 (interrupted *psbC*); lane 9: *psbC*-I2 (interruption 172 bp beyond the end of *psbC*); lane 10: *psbDC*-D1 (deletion of a large part of both copies of *psb*D and of *psb*C); lane 11: *psbD*-S197Y (site-directed mutation of His-197 of D2 into Tyr); lane 12: *psbD*-S214N (site-directed mutation of His-214 of D2 into Asn). See Table 1 for more details on the mutants.

cyanobacterial chromosome leading to kanamycin-resistant transformants can occur either by cross over in both the completely homologous 5′ and 3′ flanking regions of the *Synechocystis* 6803 *psbB* gene, or both somewhere within *psbB* in a region showing high homology between the spinach and *Synechocystis* 6803 sequences and in the 3′ flanking region of the gene. Restriction mapping of transformant DNA showed that the various kanamycin-resistant transformants differed in the length of the spinach DNA that had been integrated into the cyanobacterial chromosome and that had replaced the corresponding part of the *Synechocystis* 6803 gene: in *psbB*-C2 and *psbB*-C5 only a limited portion in the 3′ region of the gene is of spinach origin, whereas in *psbB*-C1, *psbB*-C3 and *psbB*-C4 most of the *psbB* gene is from spinach (W.F.J. Vermaas and C. Bunch, unpublished results).

The mutant *psbC*-I1 has been interrupted by a DNA fragment containing the *neo* gene at the *Sma*I site 154 bp before the 3′ end of the gene. This mutant is devoid of oxygen-evolving activity, but shows primary charge separation (W. Vermaas, H. Koike, P. Mathis and Y. Inoue, unpublished

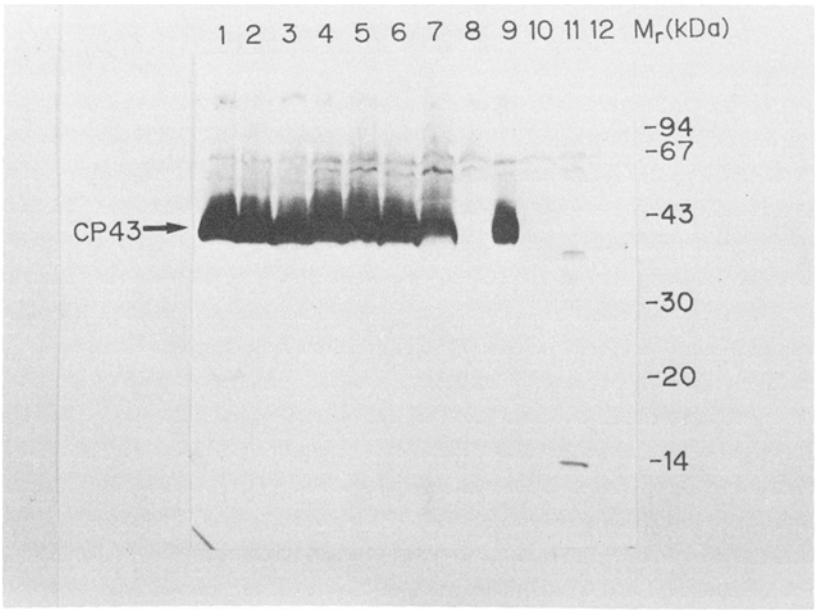

Fig. 3. Western blot of thylakoid proteins from wild type and mutant *Synechocystis* 6803 probed with rabbit polyclonal antibodies raised aginst the spinach CP43 protein. The lanes were loaded as in Fig. 2.

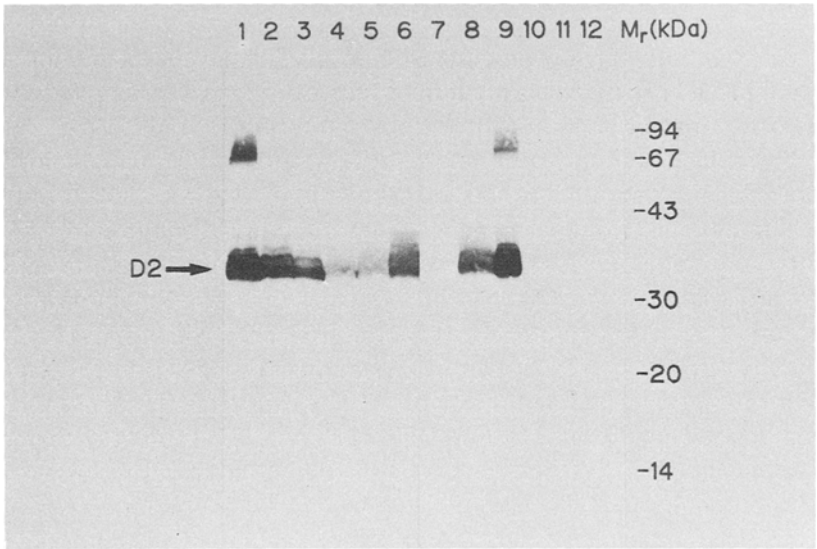

Fig. 4. Western blot of thylakoid proteins from wild type and mutant *Synechocystis* 6803 probed with rabbit polyclonal antibodies raised against the spinach D2 protein. The lanes were loaded as in Fig. 2.

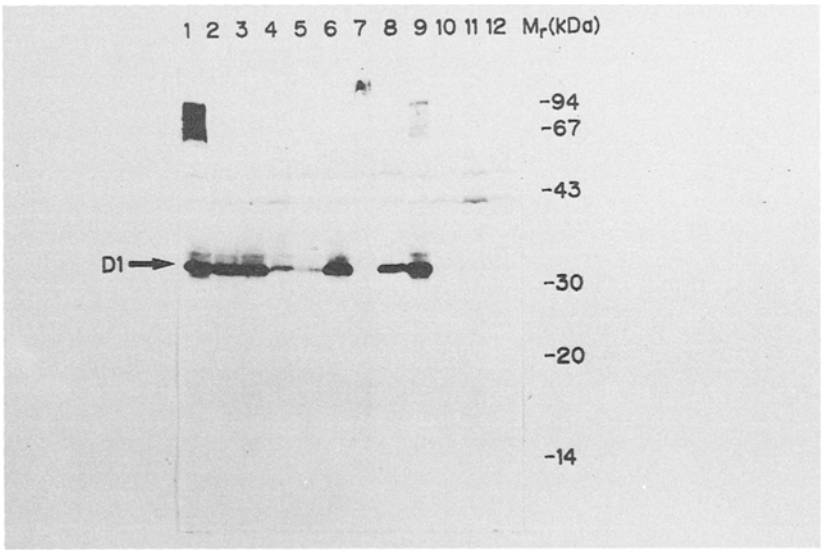

Fig. 5. Western blot of thylakoid proteins from wild type and mutant *Synechocystis* 6803 probed with rabbit polyclonal antibodies raised against the spinach D1 protein. The lanes were loaded as in Fig. 2.

data). To ascertain that the physiological effects observed were related primarily to the lack of an intact CP43 protein in the mutant, and not to synthesis inhibition of other proteins possibly encoded downstream of *psbC* under the same promoter, a DNA fragment with the *neo* gene was inserted at the *Xmn*I site 172 bp downstream of *psbC* rather than in *psbC* itself (mutant *psbC*-I2). In this case, no inhibition of oxygen-evolving activity was observed, suggesting that the inhibition of water-splitting in *psbC*-I1 indeed is due to the inhibition of synthesis of intact CP43.

Protein composition of the mutants

The thylakoid fraction from *Synechocystis* 6803 contains a large number of different proteins, making the evaluation of stained protein gels ambiguous. For this reason, blots of the protein gels were probed with antibodies specific for one of the PS II core complex components (CP47, CP43, D1 or D2). We have not yet raised antibodies against cyt b_{559}. The results are shown in Figs. 2–5.

CP47 appears to be present in the three photosynthetically competent mutants containing a hybrid *psbB* (Fig. 2; lanes 2,3 and 6) as well as in the mutants *psbC*-I1 and *psbC*-I2 (lanes 8 and 9), and some is present in the site-directed D2 mutant *psbD*-S197Y (lane 11) as was noticed before (Vermaas et al. 1987c). No CP47 was detected in the membrane fraction from the

psbD/*psbC* double-deletion strain (*psbDC*-D1) and from *psbD*-S214N (the asn-214 D2 mutant) (lanes 10 and 12, respectively). It has not yet been established whether the apparently larger CP47 signal in the three photosynthetically competent hybrid *psbB* mutants in comparison with wild type is due to a larger CP47 quantity in the membrane, or is caused by a more diffuse CP47 band in the *psbB* hybrids than in wild type under the gel conditions used. It is striking that the *psbB* chimaeric mutants that are not photosynthetically active, *psbB*-C3 and *psbB*-C4, lack detectable amounts of CP47 in the thylakoid membrane, whereas it will be pointed out in subsequent paragraphs that these mutants contain CP43, D1 and D2, although the levels of the latter two are decreased.

CP43 is present in all mutants in which *psbB* has been modified (Fig. 3; lanes 2-7). However, site-directed mutations in D2 lead to a loss of native CP43 (lanes 11 and 12), although in the *psbD*-S197Y mutant smaller polypeptides present in trace amounts are recognized by antibodies against CP43. We attribute these polypeptides to degradation products of CP43 in the thylakoid, only accumulating in detectable quantities in the *psbD*-S197Y mutant. As might be expected, interruption of the *psbC* gene or deletion of this gene leads to the absence of proteins cross-reacting with the CP43 antibody in the thylakoid membrane (lanes 8 and 10). Insertion of the *neo* gene downstream of the *psbD1*/*C* operon does not lead to loss of CP43 (lane 9) or of any of the other PS II core polypeptides.

In the mutants studied, the D2 and D1 proteins appear to be "coupled" in the sense that all mutants either contain or do not contain both D1 and D2. The D1 and D2 proteins, or polypeptides that specifically cross-react with the D1 or D2 antibodies, could not be detected in the site-directed *psbD* mutants (Figs. 4 and 5; lanes 11 and 12), the *psbD*/*C* deletion mutant (*psbDC*-D1; lane 10), and the mutant in which *psbB* had been interrupted (*psbB*-I1; lane 7). Decreased levels of both D1 and D2 appeared to be present in the photosynthetically inactive *psbB* hybrid mutants (lanes 4 and 5). In the other mutants used in this study only relatively small deviations from wild-type D1 and D2 levels in the thylakoid could be detected. However, in the wild type and (to a lesser extent) in the *psbC*-I2 mutant (lanes 1 and 9, respectively) the D1 and D2 antibodies reproducibly recognized proteins in the higher molecular weight region (possibly oligomers of D1 and/or D2), whereas this is not the case in any of the other mutants. It is not yet clear what the reason for this different behaviour may be.

In both wild type and D2-containing mutants, typically two major bands (with apparent molecular weights of 34 and 36 kDa) that cross-react with the D2 antibody can be discerned, although the photographic reproductions have caused the two bands to merge in most lanes in Fig. 4. Similar D2

doublets observed in eukaryotic photosynthetic systems have been attributed to phosphorylation of D2: the upper band represents phosphorylated D2, the lower band non-phosphorylated D2 (Delepelaire, 1984). This suggests that *Synechocystis* 6803 D2 may also be phosphorylated.

Discussion

The data presented above show that modification of one of the genes encoding a component of the PS II core complex in most cases has drastic effects on the presence and/or stoichiometry of other PS II core components. From the results shown in Fig. 2-5, the following suggestions can be made regarding the roles of the individual core proteins in stable assembly and/or synthesis of other core proteins in PS II:

1. CP47 may be needed for stable integration of D1 and D2 and for PS II activity.
2. Integration of CP43 is relatively independent of the presence of other core proteins: after interruption of *psbC* the other polypeptides are stably integrated, whereas after interruption of the *psbB* gene CP43 is present.
3. The D2 residues proposed to bind PS II cofactors appear to be of direct importance to the stability of the PS II complex. In other words, the PS II cofactors (or prosthetic groups) may play a crucial role in the assembly and stabilization of the PS II complex.
4. In each of the mutants the relative amounts of D1 and D2 in the membrane seem to be comparable.

Before elaborating on these principles, it should be noted that we cannot yet distinguish whether the effects of gene modification on the presence of other proteins in the complex result from effects at the transcriptional, translational or post-translational level, since we have no data on protein synthesis rates nor on the occurrence of mRNA coding for the PS II core components in the mutants. In the discussion mainly translational and/or post-translational control mechanisms will be considered, since these appear to occur most frequently (Jensen et al. 1986, Bennoun et al. 1986, Erickson et al. 1986). However, it should be kept in mind that other control mechanisms cannot yet be ruled out.

CP47

Until recently, CP47 was assumed to bind P680 (Nakatani et al. 1984, Yamagishi and Katoh 1985, Satoh 1986). Now, however, it seems more likely that it primarily acts as an antenna protein close to the reaction center

(see, for example, Vermaas et al. (1987d) for a review). CP47 appears to be more intricately involved with the PS II reaction center complex than CP43 is, because it is relatively easy to prepare active reaction center particles containing CP47 but without CP43 (Yamagishi and Katoh 1984), whereas the photochemically active reaction center preparation that lack CP47 is also free of CP43 (Nanba and Satoh 1987). However, the data shown here indicate that CP47 is not "just" an antenna protein. The mutants *psbB*-C3 and *psbB*-C4, both without PS II activity, contain the putative reaction center proteins D1 and D2 (although in reduced amounts as compared to wild type) as well as CP43, but do not accumulate CP47 in their thylakoids. This implies that the presence of CP47 in the membrane is required, either for structural or for functional reasons, to obtain an active PS II complex. The reduced D1 and D2 contents in mutants *psbB*-C3 and *psbB*-C4, in the absence of significant steady-state concentrations of CP47 in the membrane, may seem inconsistent with the results obtained with *psbB*-I1, in which no intact CP47 can be synthesized due to insertion of a DNA fragment encoding neomycin phosphotransferase within the coding sequence of the *psbB* gene (Vermaas et al. 1987a) and in which no membrane-bound D1 or D2 is accumulated. A possible explanation for this apparent discrepancy is that CP47, which might be synthesized in *psbB*-C3 and *psbB*-C4, but not in *psbB*-I1, is required for stable insertion of D1 and D2, but that subsequent turn over of CP47 in the PS II complex does not destabilize D1 and D2 integrated in this complex. Regardless of the validity of this hypothesis, the function of CP47 clearly is not limited to light harvesting for PS II.

It is equally obvious that CP47 is not the only factor that could regulate assembly of other polypeptides: the site-directed D2 mutants are good examples of organisms in which a specific gene modification leads to destabilization and/or inhibition of formation of other PS II components. Similarly, *psbA* (encoding D1) and *psbD* mutants from *Chlamydomonas reinhardii* lack other PS II core components (Bennoun et al. 1986, Erickson et al. 1986). However, it is difficult to compare these data with published information on other *C. reinhardii* mutants that were defective in synthesis and/or assembly of PS II components (Jensen et al. 1986) as well as in certain maize mutants (Leto et al. 1985), since the mutation sites in those mutants have not yet been established. Moreover, since some of these *C. reinhardii* mutations, as well as the maize mutation, are nuclear encoded, these mutations did not occur in any of the PS II core genes.

CP43

PS II retains its primary functions after extraction of CP43 (Yamagishi and Katoh 1984, Boska et al. 1986), which suggests that CP43 is not essential for

PS II function and primarily serves as an antenna without direct effects on the PS II reaction center. On one hand, this interpretation appears to be an oversimplification: after interruption of the *psbC* gene overall PS II activity is absent, although PS II electron transport with diphenylcarbazide as electron donor occurs (W. Vermaas, H. Koike and Y. Inoue, in preparation). On the other hand, the absence of dramatic effects of inactivation of *psbC* (and, thus, inhibition of CP43 formation) on the composition of the remainder of the PS II complex indeed suggests that CP43 is not extensively involved in assembly of the PS II core complex or in regulation of the synthesis of its components. However, it should be noted that in the CP43-less mutant the quantity of PS II core complexes in the thylakoid appears to be significantly reduced in comparison with wild type, indicating that CP43 may have a stabilizing or regulatory effect on the PS II complex. Interestingly, CP43 not only appears to be rather unimportant for assembly of the PS II complex, but other components of the complex in some cases also seem to be unimportant for incorporation of CP43 into the membrane: in the CP47 mutant *psbB*-I1, CP43 is still present in the membrane, whereas CP47, D1 and D2 are not. However, this phenomenon is not observed in the site-directed D2 mutants, where an intact CP43 is absent from the membrane, together with D1 and D2, whereas in *psbD*-S214N also CP47 is absent. A model that could explain this seeming paradox is presented at the end of the Discussion.

D2

Studies on *C. reinhardii* mutants have shown that deletion or modification of *psbA* or *psbD*, encoding D1 and D2, respectively, in most cases leads to a rapid turnover of the other PS II core proteins (Bennoun et al., 1986; Erickson et al., 1986) without a significant effect on transcription and translation. The site-directed mutations in the *psbD1* gene (and deletion of a large part of the other *psbD* gene, *psbD2*) described here may resemble the effects of modified *psbD* or *psbA* genes in *C. reinhardii*. In many respects the site-directed D2 mutations also resemble the double-deletion mutant (*psbDC*-D1), in which both *psbD* genes as well as the *psbC* gene linked to one of the *psbD* genes (*psbD1*) have been inactivated. This implies that the single amino acid change in D2 is sufficient to completely destabilize the D2 protein, under the reasonable assumption that the single base change will not have effects on translation and turnover of the mRNA or on the transcription of the gene.

The obvious question to raise at this point is why the mutation of the His-197 or His-214 residue has such dramatic effects on the stability of D2, and, hence, the other PS II core complex proteins. As was discussed in

Vermaas et al. (1987c), these histidine residues are predicted to be involved in the binding of P680, and of Q_A and Fe^{2+}, respectively, on the basis of homology with the reaction center proteins from *Rhodopseudomonas viridis* for which the crystal structure has been resolved (Deisenhofer et al. 1984 and 1985, Michel et al. 1986). This leads to the suggestion that binding of these prosthetic groups to D2 is a crucial factor in stabilization of D2, and thus PS II, in the membrane. A cofactor-binding requirement for protein stabilization is not uncommon. A similar phenomenon has been observed in antenna complexes (Bennett 1981, Youvan and Daldal 1986, Klug et al. 1986). It is not yet clear why the two site-directed mutants differ in the amount of CP43 breakdown products and CP47 in the membrane. Whatever is the mechanism, it is obvious that stable membrane-integrated D2 is crucial for assembly of other components of the PS II core complex. This is as would be expected from the proposed function of the D2 polypeptide as one of the reaction center proteins (Nanba and Satoh 1987, Vermaas et al. 1987c).

A possible scenario of complex assembly

The data discussed above indicate how much the PS II core proteins appear to depend on each other for assembly into a stable PS II complex. At this moment it is still too early to formulate a comprehensive model of the sequence of events of complex assembly in wild type and mutants, since we lack information regarding mRNA levels and translation, and protein synthesis rates. However, based on the data outlined in this paper we will describe a possible scenario for complex assembly by which the observations can be explained.

We propose that D1 and D2 first are incorporated into the membrane as an unstable pre-complex, which is stabilized by CP47 incorporation. CP43 may become part of the pre-complex even in the absence of CP47. If CP47 is absent, D1 and D2 turn over very rapidly whereas CP43 turns over much less rapidly (mutant *psbB*-I1). In the presence of CP47, none of the components turn over very rapidly, and all PS II core components accumulate in the thylakoid (wild type and mutants *psbC*-I2, *psbB*-C1, *psbB*-C2 and *psbB*-C5). Greatly reduced stability of CP47 in the thylakoid (mutants *psbB*-C3 and *psbB*-C4) leads to intermediate levels of D1 and D2. To explain this, we propose that, after formation of the membrane-integrated D1/D2/CP47 complex, turnover of CP47 does not lead to a greatly increased turn over rate of D1 and D2. This hypothesis implies that the reason for decreased D1 and D2 levels in the mutants *psbB*-C3 and *psbB*-C4 is the low concentration of integrated (or integratable) CP47 rather than increased turnover rates of integrated D1 and D2. Obviously, experiments can be designed to test these hypotheses.

Absence of CP43 has a relatively small effect on the stability of the other core components in the membrane (mutant *psbC*-I1). If synthesis of the D2 protein cannot occur, the D1/D2 complex cannot form, and therefore CP47 is not incorporated into the membrane (mutant *psbDC*-D1). If D2 cannot bind its proper prosthetic groups, the D1/D2 complex will turn over very rapidly, without allowing significant incorporation of CP47 and/or CP43. Assuming that there are differences in D1/D2 stability depending on which prosthetic groups can still be bound by D2, the differences in CP43-break-down-product and CP47 content between the two site-directed D2 mutants may thus be explained by a slightly higher stability of the D1/D2 complex of the *psbD*-S197Y mutant than of the *psbD*-S214N mutant (but both stabilities being too low to detect any D1 or D2 by Western blotting).

This scenario described above can explain the results presented in this paper as well as the results from *C. reinhardii psbA* and *psbD* mutants (Bennoun et al. 1986, Erickson et al. 1986). However, other scenarios (perhaps including "docking proteins") could be imagined that would explain the data equally well. Therefore, this model has been proposed as a basis for further experimentation and testing. It should be stressed that this model is only meant to describe *de novo* synthesis of PS II complexes, and does not address the problems raised by unequal rates of turnover of the various components of the PS II complex. Moreover, it should be kept in mind that cyt b_{559} has not been evaluated in this study, but should be taken into account since it is known to be an integral part of the PS II core complex (Satoh 1985, Arntzen and Pakrasi 1986, Nanba and Satoh 1987).

In conclusion, the *Synechocystis* 6803 PS II mutants provide valuable experimental material for studies of PS II function and assembly. However, to obtain more data for formulation and testing of detailed hypotheses on the function of each of the proteins in the assembly process, it will be necessary to generate more mutants as well as to characterize protein synthesis rates and mRNA levels of the existing mutants.

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