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Small subunits of Photosystem I reaction center complexes from *Synechococcus elongatus*. I. Is the *psaF* gene product required for oxidation of cytochrome *c*-553?

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Photosystem I (PS I) reaction center complexes isolated from the thermophilic cyanobacterium *Synechococcus elongatus* with nonionic detergents, digitonin or sucrose monolaurate, contained eight small subunit polypeptides. Two of the small polypeptides were identified by analysis of their N-terminal amino-acid sequences as the *psaF* and *psaE* gene products. Treatment with a cationic detergent, cetyltrimethylammonium bromide, resulted in depletion of five small subunits including the *psaF* gene product. Five PS I complexes isolated with an anionic detergent, sodium dodecylsulfate, contained zero to four small subunits but were all depleted of the *psaF* polypeptide. The function of the *psaF* gene product was examined by measuring reduction kinetics of flash-oxidized P-700 in the presence of different concentrations of cytochrome *c*-553. Oxidized P-700 was rapidly reduced by the reduced cytochrome in all the PS I complexes that contained, at least, the *psaC* and *psaD* polypeptides and the second-order rate constants of electron transfer from cytochrome *c*-553 to P-700 were essentially the same between PS I complexes that contained the *psaF* polypeptide and those that lost this polypeptide. Thus, the *psaF* polypeptide is not required for the bimolecular reaction between P-700 and cytochrome *c*-553. Mg^{2+} had a moderate stimulating effect on the rate of P-700 reduction whether PS I complexes were associated with the *psaF* gene product or not. The function of this subunit polypeptide is discussed.

Introduction

Photosystem I reaction center complex, which mediates light-driven electron transfer from plastocyanin or cytochrome *c*-553 to ferredoxin, is a multiprotein complex consisting of about ten subunits with molecular mass classes between 4 and 80 kDa (for reviews, see Refs. 1–3). The two large subunits of about 80 kDa, the products of *psaA* and *psaB* genes, carry the primary electron donor P-700 and the three electron acceptors, A_0, A_1 and F_X, together with several tens of antenna pigment molecules. The number of the smaller polypeptides bound to isolated PS I complexes varied depending upon preparations and analytical procedures used. Purified PS I complexes, which are active in photoreduction of NADP [4] or photooxidation of plastocyanin or cytochrome *c*-552 [5–8], or carry the full complement of the bound electron carriers [7,9], contain two to four small subunits. However, eight or more of small polypeptides were resolved by gel electrophoresis, which allows good resolution of polypeptides of less than 10 kDa [10], from PS I complexes isolated by mild procedures from higher plants and cyanobacteria. These small subunits of PS I complexes in plants and algae are partly encoded by the chloroplast DNA and partly by the nuclear DNA [2,3]. There are notable homologies of the amino-acid sequences among the corresponding subunit polypeptides of various photosynthetic organisms including cyanobacteria. The *psaC* gene product carries the iron-sulfur centers F_A and F_B [11–13] and the *psaD* and *psaF* gene products are considered to serve as the docking proteins of ferredoxin [14,15] and plastocyanin [16], respectively. However, the functions of other small polypeptides are not yet known.

The present study focuses on the function of the *psaF* gene product. Nelson and his associates, who first

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Abbreviations: CP1-a, b, c, d and e, PS I reaction center complexes isolated with SDS; CTAB, cetyltrimethylammonium bromide; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

studied the function of this subunit polypeptide (subunit III), showed that PS I preparations depleted of this subunit were unable to carry out both NADP photoreduction and cytochrome *c*-552 photooxidation [4,5]. Specific binding of the *psaF* polypeptide with plastocyanin was demonstrated by crosslinking experiments [16]. The crosslinked plastocyanin very rapidly transferred its electron to P-700$^+$ [17]. The *psaF* gene product is therefore considered to bind plastocyanin in a proper orientation for electron transfer to P-700. On the contrary, no correlation was found between the maximal rates of NADP$^+$ photoreduction and the relative amounts of the bound *psaF* polypeptide of three PS I complexes isolated from barley [18]. A mutant of *Synechocystis* sp. PCC 6803, which lacks the *psaF* gene, could grow photoautotrophically as rapidly as normal cells [19]. This result strongly suggests that the product of this gene is dispensable under physiological conditions. Thus, a question still remains whether or not the *psaF* gene product is essential for PS I electron transport.

Here, we report compositions of the small subunits of PS I reaction center complexes isolated from the thermophilic cyanobacterium *Synechococcus elongatus* with various detergents. The function of the *psaF* gene product was examined by comparing electron transfer from cytochrome *c*-553 to P-700 between PS I complexes which retained the *psaF* gene product and those which were depleted of this polypeptide. The function of the *psaE* gene product in ferredoxin reduction will be dealt with in the accompanying paper [20].

Materials and Methods

The thermophilic cyanobacterium *S. elongatus* was grown at 55°C and the thylakoid membranes were prepared as described previously [21,22].

Preparation of PS I reaction center complexes with SDS has been described previously [6]. Digitonin-PS I complexes were prepared as in [21] and further purified by gel electrophoresis with the Davis's buffer system [23]. Resolving gel contained 5% (w/v) acrylamide and 0.2% (w/v) digitonin. The slowest moving green band that contained PS I complexes were excised and the complexes were extracted from homogenized gel with 50 mM Tris-HCl (pH 7.5) and 10 mM NaCl, precipitated by centrifugation at 280 000 × *g* for 2 h and suspended in the above buffer containing 0.5 M sucrose.

Another non-ionic detergent, sucrose monolaurate, was used for preparation of larger amounts of PS I complexes. Thylakoid membranes were first treated with 0.3% (w/v) sucrose monolaurate at 30°C for 30 min and then collected by centrifugation at 270 000 × *g* for 15 min at 0°C. This procedure removed most of phycobiliproteins associated with the thylakoid membranes without any solubilization of membrane proteins. The washed membranes were incubated with 1% (w/v) sucrose monolaurate for 90 min at 30°C and centrifuged at 100 000 × *g* for 15 min at 0°C. The green supernatant obtained was applied onto a DEAE-Toyopearl column (2 × 33 cm) equilibrated with 5 mM Tris-HCl (pH 7.5). The column was washed with 50 mM Tris-HCl (pH 7.5) containing 200 mM NaCl and then with the same buffer that contained 0.05% (w/v) sucrose monolaurate and 300 mM NaCl to remove phycobiliproteins and a green fraction containing PS II. PS I complexes were eluted by increasing concentration of NaCl to 400 mM, collected by centrifugation at 220 000 × *g* for 2 h at 0°C and washed with and suspended in 50 mM Tris-HCl (pH 7.5) buffer. For depletion of the *psaF* polypeptide, the complexes (1 mg chlorophyll/ml) were further treated with 2% (w/v) CTAB at room temperature for 1 h and precipitated by centrifugation at 290 000 × *g* for 2 h. All the preparation procedures were carried out in the presence of 1 mM phenylmethylsulfonyl fluoride. In addition, the media used for detergent-treatments contained 1 mM each of EDTA and EGTA.

Polypeptide compositions of PS I complexes were analyzed by the method described in [10], which had been developed for resolution of small polypeptides. The polypeptides were transferred onto a poly(vinylidene difluoride) membrane (Millipore) and subjected to amino acid sequencing with a protein sequencer (Applied Biosystems, model 477A).

Cytochrome *c*-553 was isolated from *S. elongatus* and purified as in [24]. p*I* values of the reduced and oxidized cytochrome were determined with a Pharmacia Phast System.

Flash-induced absorption changes of P-700 were measured at 700 nm with a Union Giken single-beam spectrophotometer modified as described previously [22,25]. Flashes were fired eight times with intervals of 20 s and averaged signals were analyzed by a non-linear least-squares method with a program constructed by H.H. Reaction medium contained 50 mM Tris-HCl (pH 7.5), 2 mM methyl viologen, 10 mM sodium ascorbate, indicated amounts of cytochrome *c*-553 and thylakoid membranes or PS I preparations containing 10 μg chlorophyll ml^{-1}. When effects of Mg^{2+} were determined, the Tris buffer was replaced by 10 mM Hepes-NaOH (pH 7.5). All the measurements were carried out at about 20°C.

Chlorophyll *a* was determined by the method of Mackinney [26].

Results

Compositions of small subunits of PS I complexes

Fig. 1 shows polypeptide compositions of PS I complexes prepared from *S. elongatus* with various deter-

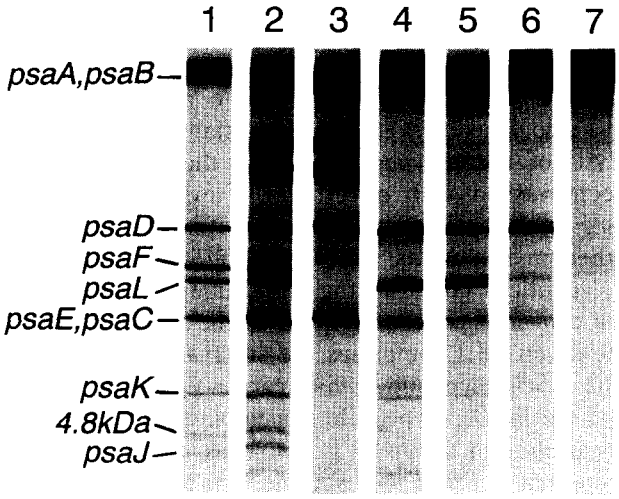

Fig. 1. Polypeptide compositions of PS I complexes prepared with different detergents. Lane 1: digitonin-complexes, lane 2: sucrose monolaurate-complexes, lane 3: CTAB-treated complexes, lane 4: CP1-a, lane 5: CP1-b, lane 6: CP1-c + d, lane 7: CP1-e.

		ref.
psaF polypeptide		
Synechococcus elongatus	DVxxLVPxKDxPAFQ	this study
Synechococcus vulcanus	DVAGLVPAKDSPAFQ	31
Synechococcus sp. PCC 6301	DVAGLTPTSESPRFI	34
Cyanophora paradoxa	DVAGLIPCSQSDAFE	a
psaE polypeptide		
Synechococcus elongatus	(M)-VQxxSKVKILxPES	this study
Synechococcus vulcanus	(M)-VQRGSKVKILRPES	31
Synechococcus sp. PCC 6301	(M)AIARGDKVRILRPES	34,35
Synechococcus sp. PCC 7002	(M)AIERGSKVKILRKES	35
Synechocystis sp. PCC 6803	(M)ALNRGDKVSIKRTES	36
Nostoc sp. PCC 8009	(M)-VQRGSKVRILRPES	35

Fig. 2. N-terminal sequences of two subunit polypeptides of digitonin-PS I complexes and their alignments with corresponding sequences of the *psaF* and *psaE* polypeptides from other cyanobacteria. (a) Unpublished data of Bryant, D.A. and Stirewalt, V.L. cited in Ref. 34. x indicates positions where unequivocal amino-acid assignments could not be made.

gents. Takahashi et al. [6] have isolated five PS I complexes (CP1-a, b, c, d and e) with different small subunit compositions by a single run of SDS gel electrophoresis. CP1-a (lane 4) was previously shown to contain three small subunits of 14, 13 and 10 kDa [6]. A fourth subunit of about 8 kDa was resolved in the gel system used here. Recently, the amino-acid sequences of the 14, 10 and 8 kDa subunits of CP1-a were analyzed. The 14 kDa [27,28] and 10 kDa [27,29] subunits are the products of the *psaD* and *psaC* genes, respectively. The N-terminal amino-acid sequence of the 8 kDa subunit [27,30] was almost identical to that of the 6.5 kDa subunit of another thermophilic cyanobacterium *S. vulcanus* [31,32] and has a weak homology to the amino-acid sequence of the *psaK* gene product of *C. reinhardtii* [33]. CP1-b had these four subunits and several contaminating polypeptides (lane 5). CP1-c and d, which had been isolated together, retained the *psaD* and *psaC* polypeptides but not the two other polypeptides (lane 6). The total absence of the small polypeptides from CP1-e was confirmed by the new gel system (lane 7).

As shown in lane 1, PS I complexes isolated with digitonin contained three more small polypeptides. Analysis of the N-terminal sequence showed that a polypeptide which migrated below the *psaD* polypeptide is the product of the *psaF* gene (Fig. 2). The sequence of the 11 amino-acid residues determined is homologous to those of the *psaF* gene products of other cyanobacteria [34] and, in particular, completely identical to that of *S. vulcanus* [31]. Note that the band intensity of the *psaC* polypeptide, relative to that of the *psaD* polypeptide, of digitonin-PS I complexes was significantly stronger than that of the corresponding band of SDS-PS I complexes (lanes 4 and 5). When digitonin-PS I complexes were run in gels containing a lower concentration of urea [20], this band was split into the *psaC* polypeptide and a more slowly moving polypeptide (see Fig. 6 of the accompanying paper [20]). The N-terminal sequence of the newly resolved polypeptide showed a significant homology to that of the *psaE* products of other cyanobacteria [34–36] and, in this case again, identical to that of *S. vulcanus* [31]. When run in parallel, electrophoretic mobilities of the

TABLE I

Compositions of the small subunits of various PS I complexes

PS I complexes	Small subunits							
	D	*F*	*L*	*E*	*C*	*K*	4.8 kDa	*J*
Digitonin complexes	+	+	+	+	+	+	+	+
Sucrose monolaurate-complexes	+	+	+	+	+	+	+	+
CTAB-treated complexes	+			+	+			
CP1-a	+		+		+	+		
CP1-b	+		+		+	+		
CP1-c + d	+				+			
CP1-e								

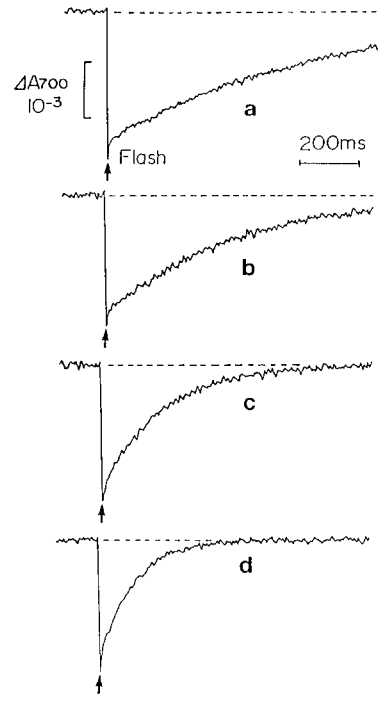

Fig. 3. Reduction kinetics of flash-oxidized P-700 in the presence of various concentrations of reduced cytochrome *c*-553. Concentrations of cytochrome *c*-553 were: (a) 1 μM; (b) 2 μM; (c) 4 μM; (d) 8 μM.

eight small subunits of *S. elongatus* complexes were identical to those of the corresponding subunits of *S. vulcanus* complexes (not shown). Three unidentified small subunits, i.e., a polypeptide which migrated below the *psaF* polypeptide and the fastest and the second fastest moving polypeptides, may be assigned to the *psaL* and *psaJ* gene products and a polypeptide corresponding to a 4.8 kDa subunit of *S. vulcanus* [31], respectively.

The polypeptide composition of PS I complexes isolated with sucrose monolaurate (lane 2) was identical to that of digitonin complexes. However, two proteolytic fragments of the large subunits appeared during preparation even in the presence of protease inhibitors. The band intensity of the *psaL* polypeptide was also weaker, suggesting a partial release or proteolysis of the polypeptide. When the complexes had been washed with a cationic detergent CTAB, the *psaF* polypeptide was extracted, together with four other small subunits, leaving the *psaD*, *psaE* and *psaC* polypeptides bound to the large subunits (lane 3). For the convenience, compositions of the small subunits of the seven PS I complexes are summarized in Table I. The *psaC* polypeptide, which carries F_A and F_B [11–13], and the *psaD* polypeptide, which binds ferredoxin [14], are present in all the PS I complexes, except for CP1-e. Note that the *psaF* polypeptide, which is considered to bind plastocyanin [15], is present only in PS I complexes prepared with the two non-ionic detergents. In the following, we determined reduction kinetics of P-700 in these PS I complexes to examine the function of the *psaF* polypeptide and other small subunits in electron transport on the oxidizing side of PS I.

Function of the psaF polypeptide

Fig. 3 shows kinetics of flash-induced absorbance changes of P-700 in digitonin-complexes which were determined in the presence of various concentrations of the reduced cytochrome *c*-553. $P\text{-}700^+$ reduction in the presence of a given concentration of the reduced cytochrome followed a pseudo-first-order kinetics and the reciprocals of the half decay times were linear to concentrations of cytochrome *c*-553 (Fig. 4A). We checked that the linear relationship hold at higher concentrations. No saturation of the rate of P-700 reduction was observed at a concentration as high as 600 μM (data not shown). The magnitude of P-700 oxidation also remained unchanged at such a high concentration of the cytochrome, indicating the absence of a very fast reduction which is indetectable with the apparatus employed. Thus, the reaction is

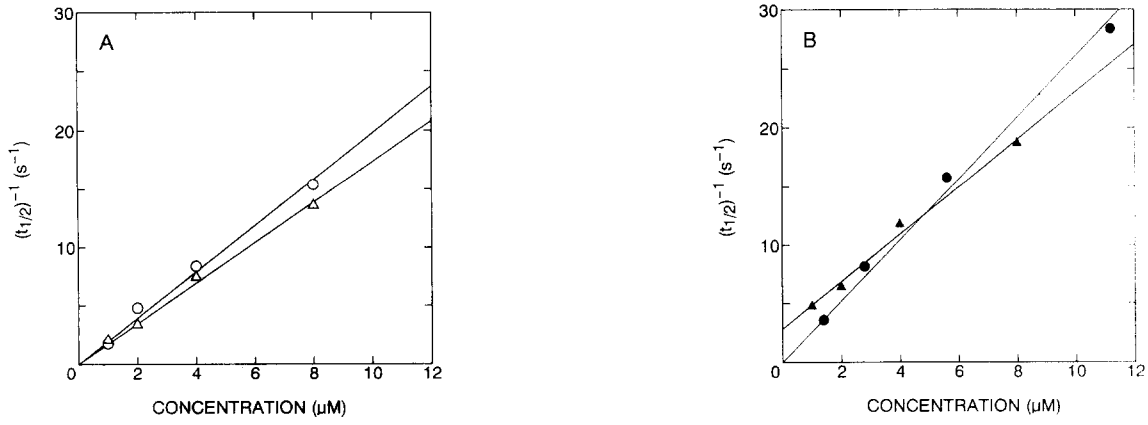

Fig. 4. Dependence of rates of P-700 reduction upon concentration of cytochrome *c*-553. (A) Open circle, thylakoid membranes; open triangles, digitonin complexes. (B) Closed circles, CP1-a; closed triangles, CTAB-treated complexes.

TABLE II

Second-order rate constants of electron transfer from cytochrome c-553 to P-700

The reaction medium contained 10 mM Hepes/NaOH (pH 7.5), 10 mM sodium ascorbate, 2 mM methyl viologen and PS I preparations equivalent to 10 μg chlorophyll/ml. Where indicated, 5 mM $MgCl_2$ was added.

Preparation	Rate constants (M^{-1} s^{-1})	
	$-Mg^{2+}$	$+Mg^{2+}$
Thylakoid membranes	$1.4 \cdot 10^6$	$3.9 \cdot 10^6$
Digitonin complexes	$1.7 \cdot 10^6$	$4.3 \cdot 10^6$
CP1-a	$1.6 \cdot 10^6$	$4.2 \cdot 10^6$

collisional in nature and the second-order rate constant was estimated as $1.2 \cdot 10^6$ M^{-1} s^{-1} (see Table II).

P-700 is located on the lumenal side of the thylakoid membranes [37]. Nevertheless, a major fraction of P-700 present in the thylakoid membranes is highly reactive with added cytochrome *c*-553 (Fig. 4A). This suggests that the membranes are leaky to small protein molecules such as cytochrome *c*-553, or have an inverted membrane orientation. The leakiness of the thylakoid preparation has previously been suggested from the total loss of cytochrome *c*-553 during preparation of the membranes [38]. Note that the rates of P-700 reduction in digitonin-PS I complexes were similar to those of intact PS I reaction center complexes present in the thylakoid membranes at all the concentrations of cytochrome *c*-553 added. This is evidence that digitonin-complexes retain an unimpaired capacity of photooxidation of cytochrome *c*-553.

PS I complexes prepared with sucrose monolaurate were also highly active in photooxidation of cytochrome *c*-553 (not shown). As stated above, CTAB-treatment of the complexes resulted in removal of the five small subunits including the *psaF* gene product. Fig. 4B shows that the second-order reaction constant of electron transfer from cytochrome *c*-553 to P-700 in the CTAB-treated complexes that contained only the three polypeptides encoded by the *psaC*, *psaD* and *psaE* genes was comparable to that in the thylakoids or digitonin-complexes. Thus, removal of the *psaF* polypeptide and the four other small subunits did not affect electron transfer from cytochrome *c*-553 to P-700. CP1-a was also depleted of the *psaF* polypeptide. The SDS-complexes also showed high rates of P-700 reduction by cytochrome *c*-553 (Fig. 4B). It is concluded, therefore, that the second-order reaction between P-700 and cytochrome *c*-553 does not require the *psaF* gene product.

Reduction of $P\text{-}700^+$ with reduced plastocyanin or cytochrome *c*-553 is strongly stimulated by addition of Mg^{2+} in various PS I preparations [8,39,40] and thylakoid membranes [40,41] because the electron donor proteins and the oxidizing domain of PS I complexes are both negatively charged. *Synechococcus* cytochrome *c*-553 is an acidic protein; p*I* values of the reduced and oxidized cytochrome were determined as 4.8 and 5.2, respectively. It was expected, therefore, that addition of Mg^{2+} results in a strong stimulation of $P\text{-}700^+$ reduction in cyanobacterial PS I complexes. However, effect of Mg^{2+} was not so large as reported in [39]. Addition of 5 mM $MgCl_2$ caused only 2- to 3-fold stimulation of P-700 reduction in the thylakoids and digitonin-complexes (Table II). This result suggests that there is a moderate electrostatic interaction between *Synechococcus* cytochrome *c*-553 and the oxidizing domain of cyanobacterial PS I complexes. It is of note that addition of Mg^{2+} induced a comparable extent of stimulation of $P\text{-}700^+$ reduction in CP1-a that lacks the *psaF* polypeptide (Table II). It is concluded, therefore, that the Mg^{2+}-effect is independent of the *psaF* polypeptide in the cyanobacterial preparations.

Discussion

PS I reaction center complexes isolated from *S. elongatus* with digitonin or sucrose monolaurate contained eight small subunits. Five small subunits were identified as the products of the *psaD*, *psaF*, *psaE*, *psaC* and *psaK* genes and three others may correspond to the *psaL* and *psaJ* polypeptides and a 4.8 kDa polypeptide of *S. vulcanus* [31]. Recently, the 9th small subunit of 3.5 kDa (*psaM* polypeptide) was found in PS I complexes from both *S. elongatus* and *S. vulcanus* [42]. The amino-acid sequences of the corresponding subunit polypeptides are highly homologous between the two cyanobacteria. Thus, the two thermophilic cyanobacteria are closely related organisms.

Digitonin- and sucrose monolaurate-complexes were highly active in photooxidation of cytochrome *c*-553 and rates of electron transfer from cytochrome *c*-553 to P-700 in the two complexes were comparable to that in intact PS I complexes present in the thylakoid membranes. However, the second-order rate constants of the electron transfer mediated by the cyanobacterial preparations were considerably smaller than the rate constants of $P\text{-}700^+$ reduction by the reduced plastocyanin in PS I preparations from higher plants, which were estimated to be in the order of 10^7 to 10^8 M^{-1} s^{-1} [40,43]. However, it is to be mentioned that the present experiments were carried out at about 20°C, which is more than 30°C below the optimum growth temperature of *S. elongatus* [38], because isolated PS I complexes were not very stable at temperatures above 40°C [44]. Thus, the difference in the rate constants should be much less than they appear when compared under conditions physiological for respective organisms.

The cationic and anionic detergents extracted differently the small subunits from PS I complexes. CTAB released five out of eight small subunits, leaving the products of the *psaC*, *psaD* and *psaE* genes bound to the complexes. The anionic detergent SDS first solubilized the *psaE*, *psaF*, *psaJ* and the 4.8 kDa polypeptides, then the *psaL* and *psaK* polypeptides and finally the two subunits encoded by the *psaC* and *psaD* genes. Thus, the small subunit encoded by the *psaF* gene was extracted by the two ionic detergents but the *psaC* and *psaD* polypeptides remained bound to the complexes. This enabled us to investigate the function of the *psaF* polypeptide in electron transfer from cytochrome *c*-553 to P-700 without any interference from a rapid back electron transport from F_X^- to P-700 which would take place if the *psaC* polypeptide is removed.

The results obtained indicate that depletion of the *psaF* polypeptide has no effect on electron transfer from cytochrome *c*-553 to P-700. CTAB-treatment of PS I complexes did not affect the reactivity of P-700 with the cytochrome. CP1-a, that lacks this polypeptide, showed a rate constant comparable to that of digitonin-complexes. It is concluded, therefore, that the *psaF* polypeptide is not required for the bimolecular reaction between P-700 and cytochrome *c*-553. The results also indicate that the products of the *psaF*, *psaL*, *psaE*, *psaK* and *psaJ* genes and the 4.8 kDa polypeptide are not essential for the reaction because they were either extracted with CTAB or absent from CP1-a. The *psaC* and *psaD* polypeptides, which remained bound to both CTAB-treated complexes and CP1-a, are located on the stromal surface of the thylakoid membranes [12,45]. Thus, none of the eight small subunits of the PS I reaction center complexes is directly involved in electron transfer between P-700 and cytochrome *c*-553.

Our results are at variance with the previous observation that photooxidation of *Euglena* cytochrome *c*-552 by PS I preparations from Swiss chard and *Chlamydomonas* was strongly inactivated by depletion of the *psaF* gene product (subunit III) [39]. Flash spectroscopy employed here provides more detailed and accurate information about the mode and rate of electron transfer from cytochrome *c*-553 to P-700 than measurement of cytochrome photooxidation with a conventional spectrophotometer under continuous illumination. However, the difference may better be attributed to different materials used, because CP1-a that has no *psaF* polypeptide was highly active in cytochrome *c*-553 photooxidation and a rate as high as 7000 μmol (mg chlorophyll)$^{-1}$ h^{-1} was recorded with continuous actinic light in the presence of a high concentration of the cytochrome [7]. Interestingly, CP1-e also showed a low but significant activity of the cytochrome photooxidation [7]. This is taken as evidence that a fast electron transfer from cytochrome *c*-553 to P-700 takes place in the total absence of the small subunits because, in the complex that lacks F_A, F_B and F_X [7] and has a diminished amount of vitamin K-1 [46], photooxidation of cytochrome *c*-553 has to compete with a very rapid back electron transfer from an earlier electron acceptor to P-700$^+$ [47].

Recently, Chitnis et al. [19] showed that a mutant of *Synechocystis sp.* PCC 6803 which lacks the *psaF* gene can photoautotrophically grow as rapidly as normal cells. This indicates that the product of this gene is not required for photosynthetic growth of the mutant cells. Based upon the observations that the growth of the mutant was further enhanced in the presence of high concentrations of $MgCl_2$ [19] and photooxidation of cytochrome *c*-553 by PS I preparations depleted of this subunit was greatly stimulated by Mg^{2+} [39], the authors suggested that the *psaF* polypeptide may be functionally replaced by Mg^{2+} [39]. However, the Mg^{2+}-effect on cytochrome photooxidation in isolated cyanobacterial PS I complexes was independent of the *psaF* polypeptide (Table II). The present study provides an alternative explanation that PS I complexes that lack this polypeptide are still competent in cytochrome photooxidation and can mediate $NADP^+$ photoreduction and ATP synthesis at rates high enough to support the normal growth of the mutant cells.

The *psaF* is considered to be the docking protein of plastocyanin [14,15,17]. Reduction kinetics of flash-oxidized P-700 in cells or chloroplasts showed a fast component with a half time of 13–17 μs and this component was ascribed to electron transfer from plastocyanin bound to this subunit polypeptide [17,48]. The occurrence of a rapid reduction of P-700 with a half-time of 10 μs or less was deduced from kinetics of redox changes of P-700, cytochrome *c*-553 and cytochrome *f* in *Synechococcus* cells at 55°C [49]. It remains to be studied whether or not the 10 μs component really exists and, if so, what the reaction mechanism of the component is.

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