Biochimica et Biophysica Acta, 1141 (1993) 52–57

BBABIO 43755

Small subunits of Photosystem I reaction center complexes from *Synechococcus elongatus*. II. The *psaE* gene product has a role to promote interaction between the terminal electron acceptor and ferredoxin

Kintake Sonoike, Hideki Hatanaka and Sakae Katoh

Department of Biology, Faculty of Science, University of Tokyo, Hongo, Bunkyo-ku, Tokyo (Japan)

(Received 2 May 1992)
(Revised manuscript received 4 August 1992)

Key words: Photosystem I; Reaction center complex; Subunit; Ferredoxin; *psaE* gene product; (*S. elongatus*)

Function of a subunit polypeptide (the *psaE* gene product) of Photosystem I (PS I) reaction center complexes was investigated by comparing the reactivity of the reduced iron-sulfur centers $(F_A/F_B)^-$ with ferredoxin among *Synechococcus* PS I complexes which had been variously depleted of this polypeptide. Ferredoxin at or below 1 μM can accept electrons from $(F_A/F_B)^-$ effectively competing with the back reaction between P-700$^+$ and $(F_A/F_B)^-$ in the thylakoid membranes and PS I complexes that contained all the eight small subunits. The high reactivity of $(F_A/F_B)^-$ with low concentrations of ferredoxin was observed in PS I complexes which contain only the products of *psaC*, *psaD* and *psaE* genes but not in complexes which carry the *psaC*, *psaD*, *psaL* and *psaK* gene products but no *psaE* gene product. Varied amounts of the *psaE* gene product were extracted by treatment with different concentrations of a cationic detergent, dodecyltrimethylammonium bromide, and 2.5 M NaCl. The solubilized polypeptide was then reconstituted to the depleted complexes. The magnitudes of the back reaction that could be suppressed by addition of ferredoxin at or below 1 μM were well correlated to the amounts of the *psaE* polypeptide remained bound or rebound to the complexes. It is concluded that the product of the *psaE* gene has a role to promote the interaction between the terminal bound electron acceptor and ferredoxin. A high autooxidizability of $(F_A/F_B)^-$ and contrasting effects of lipophilic cations and anions on the rate of the back reaction from $(F_A/F_B)^-$ to P-700$^+$ were also reported.

Introduction

The preceding paper showed that PS I reaction center complexes isolated with non-ionic detergents, digitonin or sucrose monolaurate, from the thermophilic cyanobacterium *Synechococcus elongatus* consists of two large subunits (*psaA* and *psaB* gene products) and eight small subunits (the products of *psaC*, *psaD*, *psaE*, *psaF*, *psaK*, *psaJ* and *psaL* genes and a 4.8 kDa polypeptide) [1]. The small subunit polypeptides can be depleted by the treatment with ionic detergents, CTAB and SDS. The function of the *psaF* gene product in electron transfer from cytochrome *c*-553 to P-700 was investigated by comparing the activity among PS I complexes that contained different amounts of the polypeptides.

There are several small subunits which are located on the stroma side of the thylakoid membranes, where ferredoxin reduction takes place (for reviews, see Refs. 2–4). The product of *psaC* gene carries the two iron-sulfur centers, F_A and F_B, one of which serves as a direct electron donor to ferredoxin [5,6]. Crosslinking experiments showed that the *psaD* polypeptide is the docking polypeptide of ferredoxin [7,8]. In addition the product of *psaE* gene, which is well conserved among various cyanobacteria and higher plants [2–4], is located in the close proximity of the *psaD* polypeptide on the stroma side of the thylakoid membranes [9–11]. Because an antibody raised against the *psaE* gene product strongly inhibited NADP$^+$ photoreduction, it was suggested that this polypeptide, together with the *psaD* gene product, forms a docking site for ferredoxin

Correspondence to: K. Sonoike, Department of Biology, Faculty of Science, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan.

Abbreviations: CP1-a, PS I reaction center complexes isolated with SDS; CTAB, cetyltrimethylammonium bromide; DTAB, dodecyltrimethylammonium bromide; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine; TPAC, tetraphenylarsonium chloride; TPPC, tetraphenylphosphonium chloride.

[11]. However, details of its function in ferredoxin reduction are not known yet.

The present study deals with the function of the *psaE* gene product. We examined electron transfer from F_A/F_B to ferredoxin in PS I preparations which are differently depleted of the *psaE* gene product. Reconstitution of *psaE* polypeptide to the depleted PS I complexes was also carried out. The results show that the *psaE* polypeptide is required for efficient electron transfer from the bound iron-sulfur center to the physiological concentration of ferredoxin.

Materials and Methods

PS I complexes with different polypeptide compositions were prepared from *S. elongatus* as described in the preceding paper [1].

Extraction and reconstitution of the *psaE* polypeptide were carried out as follows. PS I complexes prepared with digitonin [1] (0.4 mg Chl/ml) were treated with indicated concentrations of a cationic detergent DTAB and 2.5 M NaCl at 30°C for 1 h and precipitated by centrifugation at $356000 \times g$ for 1.5 h. For reconstitution of the small subunits solubilised, the complexes treated with 10% DTAB and 2.5 M NaCl were resuspended in 2-fold excess of the supernatant, and then dialyzed against 50 mM Tris-HCl (pH 7.5) and 10 mM NaCl for 5 h at 4°C. Because of its high critical micellar concentration (0.43%), the concentration of DTAB could be effectively reduced by this procedure. DTAB/NaCl-treated and reconstituted complexes were both once washed with the Tris buffer and resuspended in the Tris buffer containing 0.5 M sucrose.

Polypeptide compositions were analyzed by SDS gel electrophoresis according to the procedure of Ikeuchi and Inoue [12]. Because the products of the *psaE* and *psaC* genes comigrated in the original gel system containing 7.5 M urea [1], the concentration of urea was reduced to 4 M to resolve the two polypeptides.

Ferredoxin was isolated and purified from *S. elongatus* as described previously [13].

Flash-induced absorption changes of P-700 were determined at 700 nm as described in Refs. 14, 15. The basal reaction medium contained 50 mM Tris-HCl (pH 7.5), 10 mM NaCl, 10 mM sodium ascorbate, 40 μM TMPD, 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea and thylakoid membranes or PS I complexes equivalent to 10 μg Chl/ml. When necessary, 0.025% digitonin was added to ensure homogeneous dispersion of the PS I complexes. All the measurements were carried out at 20°C.

Results

The present study focuses on the function of the *psaE* gene product in reduction of ferredoxin. However, it is difficult to measure directly reduction of ferredoxin because spectral changes associated with reduction of ferredoxin are obscured by changes induced by oxidation of F_A^- or F_B^-. $NADP^+$ photoreduction has often been used to monitor the activity of PS I but this reaction is not necessarily a good measure of ferredoxin reduction. Unless a saturating concentration of the physiological electron donor, i.e., reduced plastocyanin or cytochrome *c*-553, is added, $NADP^+$ photoreduction would be limited at electron transport on the oxidizing side of PS I. We employed the following procedure to estimate the reactivity of the bound iron-sulfur center(s) with ferredoxin. Hiyama and Ke [16] have shown that, in the absence of any electron acceptor, flash-induced charge separation between P-700 and F_A/F_B (P-430) is followed by a back electron flow from $(F_A/F_B)^-$ to $P\text{-}700^+$ with a half time of 30 ms. The back reaction is suppressed by addition of an electron acceptor which can withdraw electron from the reduced terminal electron acceptor more rapidly than does $P\text{-}700^+$ [16]. Thus, the reactivity of $(F_A/F_B)^-$ with ferredoxin can be evaluated by measuring degrees of inhibition of the back reaction in the presence of various concentrations of the acceptor protein.

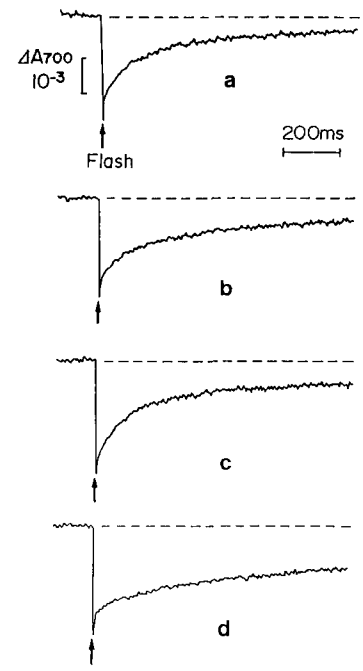

Fig. 1. Effects of ferredoxin on time courses of flash-induced redox changes of P-700 in the thylakoid membranes. Flashes of a saturating intensity were fired once every 20 s and eight signals were averaged. (a), No addition; (b), 1.6 μM ferredoxin was added; (c), thylakoid membranes were treated with 2.5% (w/v) digitonin for 30 min at room temperature; (d), (c) plus 1.6 μM ferredoxin.

Fig. 1 shows time-courses of flash-induced redox changes of P-700 in the thylakoid membranes. A small initial spike was a flash artifact. When absorption

changes were determined in the absence of any electron acceptor, reduction kinetics of P-700$^+$ consisted of two exponential components (trace a). The fast component with a half-time of 50–60 ms is ascribed to the back reaction between P-700$^+$ and $(F_A/F_B)^-$ because the component was totally eliminated by addition of methyl viologen, an efficient electron acceptor of PS I (data not shown). The slow component represents reduction of P-700$^+$ by TMPD, which was added to ensure complete reduction of P-700$^+$ during flash intervals. The half-time of the slow component decreased with increasing concentrations of TMPD (data not shown). The occurrence of the slow component is ascribed to the oxidation of a substantial fraction of $(F_A/F_B)^-$ with oxygen because this component was significantly diminished under anaerobic conditions and totally abolished by the addition of dithionite, which rapidly consumes oxygen in the reaction medium (data not shown).

The fast component was only slightly diminished by the addition of ferredoxin to the thylakoid membranes (trace b). When the membranes had been treated with digitonin, however, ferredoxin became more effective in suppressing the back reaction (trace d). Digitonin-treatment itself had no effect on the reduction kinetics of P-700$^+$, although the signal size increased due to a diminished flattening effect (trace c). Fig. 2 shows effects of different concentrations of ferredoxin on the fast component of P-700$^+$ reduction in untreated and digitonin-treated membranes. The fast component was suppressed only to a small extent even at the highest concentration of ferredoxin added in untreated thylakoid membranes but, after digitonin-treatment, a large

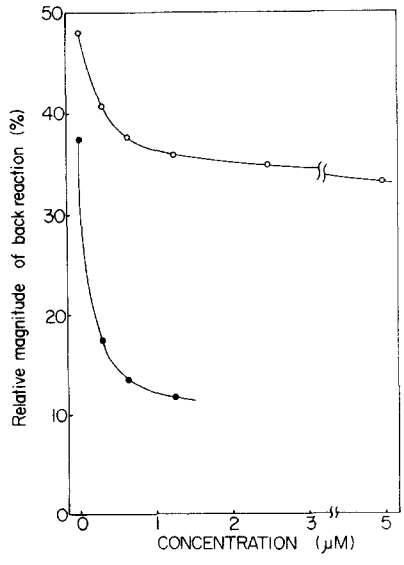

Fig. 2. Effects of different concentrations of ferredoxin on the back reaction between P-700$^+$ and $(F_A/F_B)^-$. Open circles, untreated thylakoid membranes; closed circles, thylakoid membranes treated with 2.5% digitonin for 30 min.

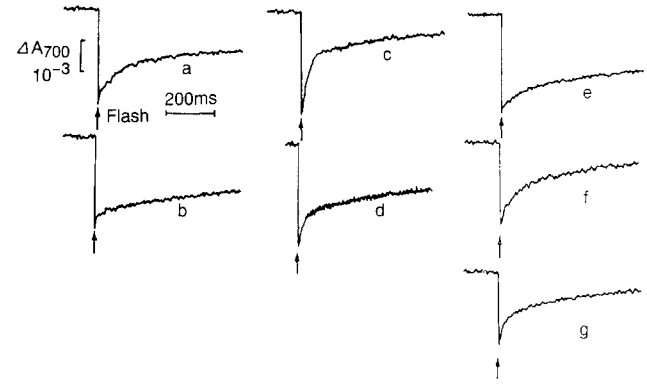

Fig. 3. Reduction kinetics of P-700$^+$ in various PS I complexes and effects of ferredoxin on the fast reduction component. (a) digitonin-PS I complexes; (b) (a) plus 1.2 μM ferredoxin; (c) PS I complexes prepared with sucrose monolaurate and then treated with CTAB; (d) (c) plus 1.2 μM ferredoxin; (e) SDS-PSI complexes (CP1-a); (f) (e) plus 100 mM TPPC; (g) (f) plus 1.2 μM ferredoxin.

inhibition of the back reaction by ferredoxin occurred. Note that the back reaction was effectively inhibited by addition of ferredoxin in the submicromolar concentration region in both untreated and digitonin-treated membranes. This indicates that low concentrations of ferredoxin can serve as an efficient electron acceptor of PS I.

Digitonin-PS I complexes, which carry all the eight small subunits [1], also showed biphasic reduction kinetics of P-700 (Fig. 3, trace a). The fast component was diminished by addition of 1.2 μM ferredoxin (trace b). Similar results were obtained with PS I complexes isolated with sucrose monolaurate (data not shown). Further treatment of the PS I complexes with a cationic detergent CTAB resulted in a marked acceleration of the fast component (trace c). The accelerated reduction of P-700$^+$ was significantly diminished by addition of ferredoxin (trace d).

CP1-a, a PS I complex isolated with SDS [1,17], showed reduction kinetics of P-700$^+$ with a diminished fast decay component (Fig. 4, trace e, also see Ref. 15). However, the following findings enabled us to examine the effect of ferredoxin on the back reaction in this preparation. SDS is an anionic detergent, while CTAB carries a positive charge. Thus, the contrasting effects of the two detergents on the back reaction may be related to the opposite charges the detergent molecules carry. In fact, two lipophilic cations, TPAC and TPPC increased the magnitude of the fast component at concentrations above 1 mM, whereas a lipophilic anion, tetraphenylboron, strongly suppressed the fast component of P-700$^+$ reduction (Fig. 4). $MgCl_2$ and NaCl were without effect (data not shown). The fast component was markedly enhanced when 100 mM TPPC was added to CP1-a (Fig. 3, trace f) and the component thus enhanced was appreciably suppressed by ferredoxin (trace g).

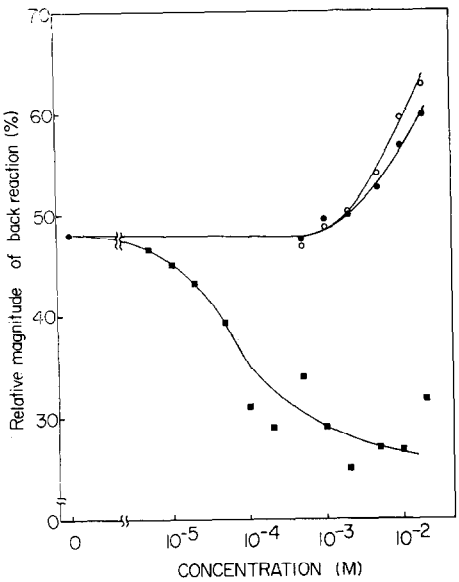

Fig. 4. Effects of lipophilic ions on the magnitude of the back reaction in the thylakoid membranes treated with 2.5% (w/v) digitonin. Open circles, TPAC; closed circles, TPPC; closed squares, sodium tetraphenylboron.

Fig. 5 shows effects of different concentrations of ferredoxin on the fast component in CTAB-treated complexes and CP1-a. After CTAB-treatment, about 75% of P-700$^+$ was reduced through the back reaction with $(F_A/F_B)^-$ and low concentrations of ferredoxin effectively suppressed the charge recombination. However, the cation-enhanced fast component of CP1-a decreased monotonously with increasing concentration of ferredoxin without showing a sharp inhibition of the back reaction at low concentrations of ferredoxin (Fig. 5, also see Fig. 3, trace g). As described in the preceding paper [1], CTAB-treated PS I complex carries only three small subunits, the *psaC*, *psaD* and *psaE* gene

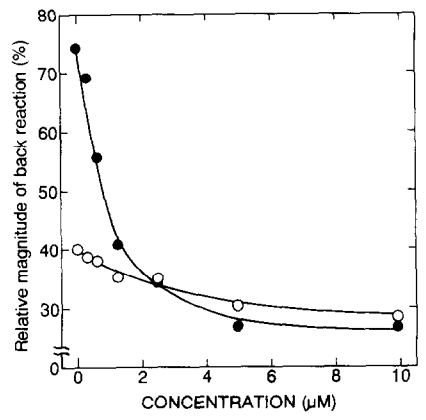

Fig. 5. Effects of different concentrations of ferredoxin on the fast component of P-700$^+$ reduction in PS I complexes treated or isolated with ionic detergents. Closed circles, CTAB-treated complexes; open circles, CP1-a plus 100 mM TPPC.

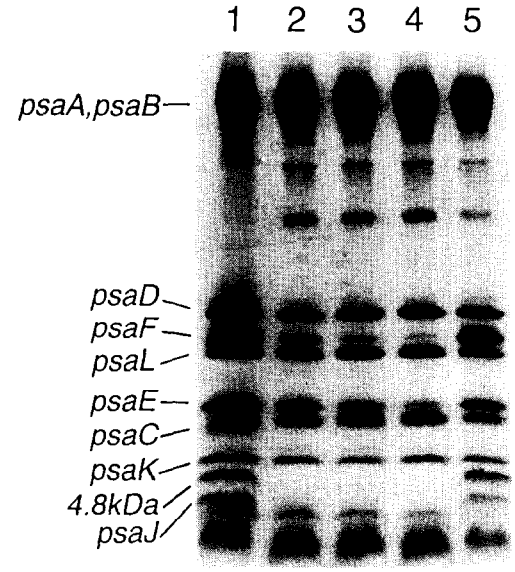

Fig. 6. Effects of DTAB/NaCl treatment on the subunit composition of digitonin-PS I complexes. Lane 1, untreated digitonin complexes; lane 2, 3 and 4, digitonin-PS I complexes treated with 2.5%, 5% and 10% DTAB in the presence of 2.5 M NaCl; lane 5, digitonin-PS I complexes treated with 10% DTAB plus 2.5 M NaCl and then reconstituted with the solubilized polypeptides. The two bands appeared in the upper region of the gel after DTAB/NaCl treatment were proteolytic products of the large subunits.

products, whereas CP1-a contains the *psaC*, *psaD* and other polypeptides but no *psaE* gene product. Thus, the results suggest that the efficient interaction between $(F_A/F_B)^-$ and ferredoxin occurs only in PS I complexes with the *psaE* polypeptide attached.

The following experiments were carried out to examine this possibility. Lane 1 of Fig. 6 shows the location of the eight small subunit polypeptides of untreated digitonin-PS I complexes in the gel. The *psaC* and *psaE* polypeptides, which had comigrated in gels containing 7.5 M urea [1], were well separated from each other in gels containing 4 M urea. Various subunit polypeptides were solubilized by this treatment with a cationic detergent DTAB in the presence of 2.5 M NaCl (lanes 2–4). Treatment with 2.5% DTAB resulted in the total solubilization of the 4.8 kDa polypeptide and the *psaJ* gene product (lane 2). The same concentration of the detergent extracted largely the *psaF* polypeptide but only partly the *psaE* polypeptide. The *psaE* polypeptide was extracted at higher concentrations of the detergent (lanes 3 and 4). The presence of 2.5 M NaCl was essential for the efficient extraction of *psaE* gene product. Other polypeptides were not extracted by these treatments. Lane 5 indicates that the *psaE* polypeptide was partially reconstituted to the PS I complexes which had been treated with 10% DTAB and 2.5 M NaCl, when the concentrations of the detergent and NaCl had been reduced as described in the Materials and Methods

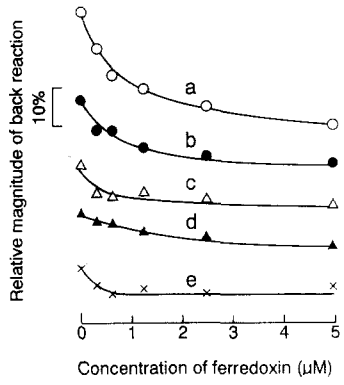

Fig. 7. Effects of different concentrations of ferredoxin on the reduction kinetics of P-700$^+$ in the digitonin PS I-complexes, of which polypeptide compositions were determined in Fig. 6. Because DTAB/NaCl complexes tended to aggregate, 0.025% digitonin was added to the reaction medium to ensure homogeneous dispersion of the complexes. For the sake of comparison, curves are vertically displaced. Curve a, untreated digitonin-PS I complexes; curve b, c and d, digitonin-PS I complexes treated with 2.5%, 5% and 10% DTAB in the presence of 2.5 M NaCl; curve e, digitonin-PS I complexes treated with 10% DTAB plus 2.5 M NaCl and then reconstituted with the solubilized polypeptides.

section. Rebinding of the other solubilized polypeptides was also observed.

Fig. 7 shows that effects of low concentrations of ferredoxin on the fast component of P-700 reduction became progressively less significant as the DTAB concentration was raised and became negligible at the highest concentration of the detergent used (curves a–d). The magnitudes of the fast component that could be diminished by the addition of ferredoxin in the 1 μM region are well correlated with the amounts of the *psaE* polypeptide, but not with that of the 4.8 kDa or the *psaJ* polypeptide, remaining bound to the complexes. Note that low concentrations of ferredoxin became again effective in suppressing the back reaction when the solubilized polypeptides were reconstituted (curve e) and the magnitude of the restoration was well correlated with the amount of the *psaE* polypeptide rebound to the complexes. Involvement of *psaF* polypeptides in the ferredoxin reduction can be excluded because of its localization on the lumenal surface of the thylakoid membranes. It is concluded, therefore, that the *psaE* polypeptide is required for the interaction of the terminal bound acceptor with low concentration of ferredoxin.

Discussion

The present study indicate that the product of the *psaE* gene plays an important role in ferredoxin reduction. The fast component of P-700 reduction, which represents the back electron transfer from F_A^- or F_B^- to P-700$^+$, in the thylakoids and PS I complexes containing the *psaE* polypeptide was strongly suppressed by addition of ferredoxin in the submicromolar concentration region. The high-affinity interaction between $(F_A/F_B)^-$ and ferredoxin disappeared when the *psaE* polypeptide had been depleted but reappeared on reconstitution of the polypeptide to depleted complex. PS I preparations that lack this polypeptide but contain the *psaC* and *psaD* gene products are still active in ferredoxin phototreduction as judged from the observation that the magnitude of the back reaction in CP1-a decreased gradually at higher concentrations of ferredoxin. These observations indicate that the *psaD* gene alone can mediate electron transfer from ferredoxin to $(F_A/F_B)^-$ but the *psaE* gene product is required for efficient oxidation of $(F_A/F_B)^-$ by low concentrations of ferredoxin.

The *psaC* polypeptide carries F_A and F_B and ferredoxin is considered to bind to the *psaD* polypeptide to accept electron from $(F_A/F_B)^-$ [7,8]. Crosslinking experiments indicated that the *psaE* polypeptide is located very close to the *psaD* polypeptide [9–11]. There are several possible explanations for the function of the *psaE* polypeptide. First, the binding of the *psaE* polypeptide to the *psaD* polypeptide alters the conformation of the *psaD* polypeptide so as to increase its binding affinity to ferredoxin. Second, the *psaE* polypeptide itself provides an additional binding site for ferredoxin. Third, the *psaE* polypeptide affects the conformation of the *psaC* polypeptide in a way to enhance interaction between F_A/F_B and ferredoxin. Inhibition of NADP$^+$ photoreduction by the antibody against the *psaE* polypeptide [11] favors the second possibility. More experiments are required to elucidate the mechanism of the function of this subunit polypeptide.

Targeted mutagenesis technique has been used to inactivate the *psaE* gene in *Synechocystis* sp. PCC 6803 [18] and *Synechococcus* sp. PCC 7002 [19]. The two deletion mutants could photoautotrophically grow at rates not much different from that of wild-type cells. It has been suggested, but not yet proved, that the *psaE* polypeptide is functionally replaced by a 15 kDa subunit polypeptide which is produced more abundantly in the mutant than in the wild-type cells [20]. Our results indicate that depletion of this polypeptide affects the affinity of interaction between $(F_A/F_B)^-$ and ferredoxin but does not abolish photoreduction of ferredoxin. Thus, the mutant PS I complexes would be still active in NADP$^+$ photoreduction. In this respect, it is interesting to examine whether the mutant cells contain higher concentrations of ferredoxin than do the wild-type cells.

The back reaction was effectively suppressed at low concentrations of ferredoxin in CTAB-treated complexes. This indicates that the small polypeptide other than the *psaC*, *PsaD* and *psaE* gene products are not

necessary for efficient electron transfer from $(F_A/F_B)^-$ to physiological concentrations of ferredoxin.

The present work also revealed several interesting features of cyanobacterial PS I. First, the rate of the back electron transfer from $(F_A/F_B)^-$ to P-700$^+$ was strongly affected by lipophilic ions. Lipophilic cations accelerated the fast component of P-700 reduction, whereas the back reaction was diminished in the presence of lipophilic anions. Hydrophilic ions such as Mg^{2+} was totally ineffective. It is suggested, therefore, that lipophilic ions enter into a lipophilic domain near P-700 and hence the charge recombination between P-700$^+$ and $(F_A/F_B)^-$ is electrostatically accelerated by cations and diminished by anions.

Second, the reduced terminal electron acceptor of PS I was found to be highly autooxidizable. Reduction kinetics of P-700$^+$ suggests that $(F_A/F_B)^-$ transfers its electron to oxygen at a rate comparable to that of the back electron transfer to P-700$^+$. The terminal electron acceptor was similarly reactive with oxygen in the thylakoid membranes from another thermophilic cyanobacterium, *Synechococcus vulcanus* (Sonoike, K., unpublished data). The autooxidizability of $(F_A/F_B)^-$ appears to vary depending upon organisms and preparations examined [16,21,22]. However, the leakage of electrons to oxygen should be negligible in cells because $(F_A/F_B)^-$ is oxidized by physiological concentrations of ferredoxin much more rapidly than by oxygen.

Third, a major population of the terminal electron acceptor of PS I, which is located on the stroma side of the thylakoid membranes, was found to be inaccessible to externally added ferredoxin in the cyanobacterial thylakoids. Only when the membrane structure had been disrupted with digitonin was the back reaction between P-700$^+$ and $(F_A/F_B)^-$ largely suppressed by the addition of ferredoxin. In this respect, of interest is the observation that P-700, which is located on the lumenal surface of the membranes is mostly accessible to added cytochrome *c*-553 [1]. Combined, these observations suggest an inverted orientation of the thylakoid membranes. If so, the cyanobacterial membranes will serve as interesting material for the investigation of topographical properties of the thylakoid membranes.

Acknowledgements

This work was supported in part by grants for Scientific Research from the Ministry of Education, Science and Culture, Japan.

References

1 Hatanaka, H., Sonoike, K., Hirano, M. and Katoh, S. (1993) Biochim. Biophys. Acta, 1141, 45–51.
2 Scheller, H.V. and Møller, B.L. (1990) Physiol. Plant. 78, 484–494.
3 Golbeck, J.H. and Bryant, D.A. (1991) in Current Topics in Bioenergetics (Lee, C.P., ed.), Vol. 16, pp. 83–177, Academic Press, New York.
4 Bryant, D.A. (1992) in Current Topics in Photosynthesis (Barber, J., ed.), Vol. 11, Elsevier, Amsterdam, in press.
5 Wynn, R.M. and Malkin, R. (1988) FEBS Lett. 229, 293–297.
6 Oh-oka, H., Takahashi, Y., Kuriyama, K., Saeki, K. and Matsubara, H. (1988) J. Biochem. 103, 962–968.
7 Zanetti, G. and Merati, G. (1987) Eur. J. Biochem. 169, 143–146.
8 Zilber, A.L. and Malkin, R. (1988) Plant Physiol. 88, 810–814.
9 Oh-oka, H., Takahashi, Y. and Matsubara, H. (1989) Plant Cell Physiol. 30, 869–875.
10 Andersen, B., Koch, B., Scheller, H.V., Okkels, J.S. and Møller, B.L. (1990) in Current Research in Photosynthesis, (Baltscheffsky, M., ed.), Vol. 2, pp. 671–674, Kluwer, Dordrecht.
11 Andersen, B., Koch, B. and Scheller, H.V. (1992) Physiol. Plant. 84, 154–161.
12 Ikeuchi, M. and Inoue, Y. (1988) Plant Cell Physiol. 29, 1233–1239.
13 Koike, H. and Katoh, S. (1979) Plant Cell Physiol. 20, 1157–1161.
14 Hirano, M., Satoh, K. and Katoh, S. (1980) Photosynth. Res. 1, 146–162.
15 Sonoike, K. and Katoh, S. (1988) Biochem. Biophys. Acta 935, 61–71.
16 Hiyama, T. and Ke, B. (1971) Arch. Biochem. Biophys. 147, 99–108.
17 Takahashi, Y., Koike, H. and Katoh, S. (1982) Arch. Biochem. Biophys. 219, 209–218.
18 Chitnis, P.R., Reilly, P.A., Meidel, M.C. and Nelson, N. (1989) J. Biol. Chem. 264, 18374–18380.
19 Bryant, D.A., Rhiel, E., de Lorimier, R., Zhou, J., Stirewalt, V.L., Gasparich, G.E., Dubbs, J.M. and Snyder, W. (1990) in Current Research in Photosynthesis (Baltscheffsky, M., ed.), Vol. 2, pp. 1–9, Kluwer, Dordrecht.
20 Franzén, L.-G., Frank, G., Zuber, H. and Rochaix, J.-D. (1989) Mol. Gen. Genet. 219, 137–144.
21 Mannan, R.M., Whitmarsh, J., Nyman, P. and Pakrasi, H.B. (1991) Proc. Natl. Acad. Sci. USA 88, 10168–10172.
22 Nyhus, K.J., Ikeuchi, M., Inoue, Y., Whitmarsh, J. and Pakrasi, H.B. (1992) J. Biol. Chem., in press.