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PsaL subunit is required for the formation of photosystem I trimers in the cyanobacterium *Synechocystis* sp. PCC 6803

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When membranes of the wild type strain of the cyanobacterium *Synechocystis* sp. PCC 6803 were solubilized with detergents and fractionated by sucrose-gradient ultracentrifugation, photosystem I could be obtained as trimers and monomers. We could not obtain trimers from the membranes of any mutant strain that lacked PsaL subunit. In contrast, absence of PsaE, PsaD, PsaF, or PsaJ did not completely abolish the ability of photosystem I to form trimers. Furthermore, PsaL is accessible to digestion by thermolysin in the monomers but not in the trimers of photosystem I purified from wild type membranes. Therefore, PsaL is necessary for trimerization of photosystem I and may constitute the trimer-forming domain in the structure of photosystem I.

Photosystem I; Photosynthesis; Organization; Cyanobacteria; *Synechocystis* sp. PCC 6803

1. INTRODUCTION

Photosystem I is one of the membrane–protein complexes of the photosynthetic apparatus of cyanobacteria and plants. It accepts electrons from plastocyanin or cytochrome c_{553} on the *p*-side (lumenal) of the membrane and donates them to ferredoxin or flavodoxin on the *n*-side (cytoplasmic or stromal). PSI has been isolated from higher plants, algae, and cyanobacteria and is remarkably conserved regarding its function, structure, and subunit composition [1,2]. Purified PSI typically contains at least eleven polypeptides, approximately 100 chlorophyll *a* molecules, a pair of phylloquinones and three 4Fe–4S clusters [1]. Subunits PsaA and PsaB are two homologous chlorophyll-binding polypeptides that contain the electron transfer centers P700, A_0 and A_1, and the 4Fe–4S center F_X [1,3]. PsaC contains the remaining iron–sulfur centers, F_A and F_B [4]. The remaining subunits of PSI do not contain any electron transfer components and are accessory in their function.

Organization of proteins in PSI has been inferred from their hydropathy profiles, the nature of their transit peptides, biochemical experiments involving protease accessibility, antibody-epitope mapping, chemical cross-linking and reconstitution [1,2]. Some domains of the PsaD, PsaE and PsaL subunits of PSI from higher plants are exposed to proteases [5–7]. The large subunits (PsaA and PsaB) and some low molecular weight subunits (PsaL, PsaK, PsaI, PsaJ, and PsaM) are integral membrane proteins with hydrophobic transmembrane domains [8]. PsaF is exposed on the *p*-side of the membrane. PsaD, PsaC and PsaE are on the *n*-side of photosynthetic membrane. Cross-linking and in vitro reconstitution experiments reveal that PsaD, PsaE and PsaC are in contact with each other and a considerable part of PsaC is probably buried under PsaD and PsaE [6,9–11]. Electron microscopy and electron diffraction studies suggest that these three proteins form a protruding cap on the stromal surface of the PSI complex [12,13].

The cyanobacterium *Synechocystis* sp. PCC 6803 provides an attractive system for studying the structure–function relationship in PSI. Simpler genetic organization, natural transformability the capacity to carry out homologous recombination, and the ability to grow under heterotrophic conditions make targeted mutagenesis of photosynthetic proteins possible in this species [14]. We are studying the functions of the accessory subunits of PSI through targeted mutagenesis of the genes that encode these subunits in *Synechocystis* sp. PCC 6803. Previously we cloned and characterized *psaD*, *psaE*, *psaF*, *psaJ* and *psaL* and then generated mutant strains in which these genes have been inactivated [15–19]. In the present study, we investigated the presence of trimeric quaternary structure of PSI in the membranes of these mutants. We report the absence of PSI trimers in the detergent-solubilized membranes of PsaL-less strains and show that PsaL is susceptible to proteolysis in monomers but not in trimers of in wild type PSI.

2. MATERIALS AND METHODS

Radioactive chemicals were purchased from NEN Corp. All enzymes and reagents used for molecular cloning were obtained from

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Promega Biotech (Madison, WI). Detergents were from Calbiochem (La Jolla, CA). The other chemicals and antibiotics were purchased from Sigma or Fisher Biotech. The antibody specific to the carboxyl terminus of PsaB was kindly provided by Dr. James Guikema, Kansas State University [20]. The cyanobacterial strains used in this study are described in Table I.

Synechocystis sp. PCC 6803 cultures of both the wild-type and mutant strains were grown in BG11 medium containing 5 mM glucose and appropriate antibiotics [21]. Cells were harvested during the late exponential phase of growth and suspended in 0.4 M sucrose, 10 mM NaCl, 200 μM phenylmethyl sulfonylfluoride, and 5 mM benzamidine, and 50 mM Tris, pH 8.0. Equal volume of 150–200 μm glass beads was added, and the cells were broken with a bead beater (Biospec). Photosynthetic membranes were separated from unbroken cells, washed with, and then resuspended in 0.4 M sucrose, 10 mM NaCl, and 50 mM Tris, pH 8.0. The membranes were incubated typically with dodecyl-β-D-maltoside (w/w chlorophyll:detergent ratio of 1:15) for 15 min on ice and then centrifuged for 15 min at 20,000 × g at 4°C. The solubilized membranes were layered on a step-gradient made up of 10%, 15%, 20%, 25% and 30% sucrose containing 10 mM MOPS (pH 7.0) and 0.01% dodecyl-β-D-maltoside. The samples were centrifuged at 200,000 × g for 16 h at 4°C. Chlorophyll concentrations in membranes and PSI fractions were determined in 80% (v/v) acetone according to [22]. The photosynthetic membranes or PSI fractions were stored at −20°C until use.

For protease accessibility studies, monomers and trimers of PSI from wild-type membranes (100 μg chlorophyll/ml) were incubated with thermolysin at a concentration of 50 μg protease per mg of chlorophyll in the presence of 1 mM $CaCl_2$ at 37°C for different durations. The reactions were terminated with 20 mM EDTA and the samples were solubilized for 1 h at room temperature in the presence of 1% SDS and 0.1% β-mercaptoethanol. Proteins were separated by Tricine-urea-SDS-PAGE according to [23] on slab gels containing 14% acrylamide and 6 M urea. To determine relative levels of different subunits of PSI complexes, the gels were stained with Coomassie blue, destained, and scanned with a personal laser densitometer (Molecular Dynamics). For Western blotting, proteins were transferred to nitrocellulose membranes, probed with an antibody against the C-terminus of PsaB, and immunodetected using enhanced chemiluminescence kit (Amersham).

3. RESULTS AND DISCUSSION

The quaternary structure of PSI may involve the formation of trimers. Several groups have isolated and characterized trimers of PSI from cyanobacteria, plants and a prochlorophyte [24–32]. Steady-state polarized light spectroscopy of the monomers and trimers of PSI has revealed remarkable similarity between the two, except for the amplitude of a spectral component at long wavelength. Despite some supporting data, the existence of trimers in vivo is difficult to demonstrate [33]. We optimized conditions for obtaining monomers and trimers of PSI by sucrose-gradient centrifugation. The choice of detergent used for solubilization of membranes and the ratio of detergent to membranes influenced the yield of the trimeric form of PSI. We tested Triton X-100, hexyl-, heptyl-, octyl-, nonyl-, and decyl-β-glucopyranoside, deoxycholate, zwittergent detergents 3-08, 3-10, 3-12, and 3-16, and dodecyl-β-D-maltoside for their ability to resolve PSI from membranes into monomeric and trimeric forms. Dodecyl-β-D-maltoside was the most suitable detergent to dissolve membranes with minimal disturbance to the quaternary structure of PSI. The ratio (w/w) of chlorophyll to detergent during solubilization of membranes affected the proportion of PSI that could be isolated as trimers; the optimal ratio for the photosynthetic membranes of *Synechocystis* sp. PCC 6803 was found to be 1:15. After incubating with the detergent for 15 min on ice, the membranes (in 5% sucrose, 50 mM Tricine, 10 mM NaCl) were centrifuged at 20,000 × g for 15 min and the supernatant was layered on a 10–30% step-gradient of sucrose. Centrifugation in 13 ml tubes at 200,000 × g for 16 h resolved the pigmented complexes of the photosynthetic membranes into distinct bands (Fig. 1A). Absorption spectra of the pigmented bands indicated that the upper orange band contained carotenoid-proteins. The middle and lower green bands contained chlorophyll. The gradients were fractionated into 1 ml parts and their chlorophyll contents were determined. The middle green band contained approximately 50% of total chlorophyll that was layered on the gradient, while the heavier green band contained the remaining chlorophyll (Fig. 1B). All fractions containing chlorophyll con-

Table I

Strains of *Synechocystis* sp. PCC 6803 that were used in this study

Strain	Description	Reference
WT	Glucose-tolerant wild-type strain	[14]
AEK2	The *psaE* gene replaced by a gene for kanamycin resistance	[16]
ADC4	The *psaD* gene replaced by a gene for chloramphenicol resistance	Chitnis, unpublished
AFK6	The *psaF* gene replaced by a gene for kanamycin resistance. The *psaJ* gene transcriptionally inactivated	[17]
ALC7-3	The *psaL* gene replaced by a gene for chloramphenicol resistance	[18]
DE	The *psaE* and *psaD* genes replaced by genes for kanamycin and chloramphenicol resistance, respectively	Chitnis, unpublished
DF	The *psaF* and *psaD* genes replaced by genes for kanamycin and chloramphenicol resistance, respectively. The *psaJ* gene transcriptionally inactivated	Chitnis, unpublished
EF	The *psaF* and *psaE* genes replaced by genes for kanamycin and chloramphenicol resistance, respectively. The *psaJ* gene transcriptionally inactivated	Xu, Chitnis, Chitnis, unpublished
EL	The *psaE* and *psaL* genes replaced by genes for kanamycin and chloramphenicol resistance, respectively	Xu, Chitnis, Chitnis, unpublished
FL	The *psaF* and *psaL* genes replaced by genes for kanamycin and chloramphenicol resistance, respectively. The *psaJ* gene transcriptionally inactivated	Xu, Chitnis, Chitnis, unpublished

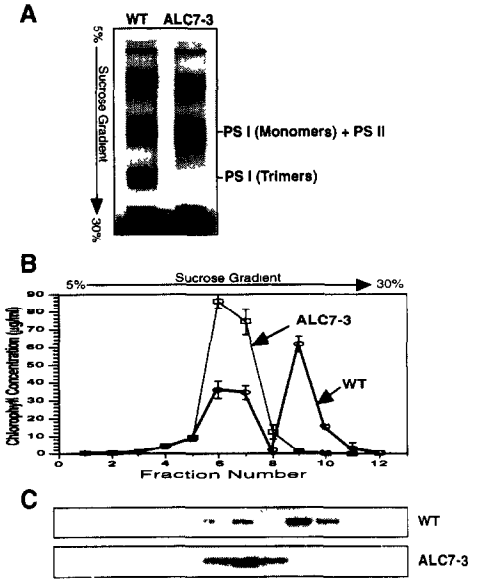

Fig. 1. Resolution of monomeric and trimeric forms of photosystem I by sucrose-gradient centrifugation. Photosynthetic membranes containing 200 μg chlorophyll in 5% sucrose were incubated on ice for 15 min with dodecyl-β-D-maltoside (1:15 chlorophyll/detergent ratio), centrifuged at 20,000 × g for 15 min at 4°C. The solubilized membranes were layered on a 10–30% sucrose gradient containing 20 mM MOPS and 0.01% dodecyl-β-D-maltoside. The samples were centrifuged for 16 h at 200,000 × g (panel A). The gradients were fractionated into 1 ml parts and chlorophyll content of each fraction was determined (panel B). The values are an average of three independent experiments and the bars represent standard deviation. The presence of PSI in the chlorophyll-containing fractions was confirmed by Western blot analysis using an antibody against the PsaB subunit of PSI (panel C). The antibody–antigen reaction was detected using a horseradish-peroxidase conjugated antibody and enhanced chemiluminescence substrates for horseradish peroxidase.

tained PSI, as evident from SDS-PAGE analysis (data not shown) and Western blotting (Fig. 1C). The upper green fraction also contained photosystem II (data not shown). When a mixture of molecular weight markers was layered on the top of gradients and centrifuged, the median positions of the markers were: bovine serum albumin (66 kDa) in fraction #4, alcohol dehydrogenase (150 kDa) in fraction #5, β-amylase (200 kDa) in fraction #6, bovine thyroglobulin (669 kDa) in fraction #9 and blue dextran (2000 kDa) at the bottom. The estimated molecular masses of monomers and trimers of PSI are 235–300 kDa and 670–750 kDa, respectively [27,28,31]. Thus solubilization of the wild-type membranes by dodecyl-β-D-maltoside resulted into lighter and heavier forms of PS I which presumably represented monomers and trimers of PSI. Similar treatment of membranes from ALC7-3, the PsaL-less mutant strain, resulted in only one green band on sucrose-gradients that corresponded to the upper green band in the tubes containing the wild-type PSI. When ALC 7-3 membranes were incubated with the detergent and centrifuged on sucrose gradients, the fractions that normally contained the trimers of PSI in wild-type membranes did not contain any chlorophyll or PsaB protein (Fig. 1). Therefore trimers of PSI could not be obtained by the procedures that yielded maximal amounts of trimers from the wild type membranes. We varied the detergent to chlorophyll ratio during solubilization of membranes and used other detergents listed before, but failed to obtain PSI trimers from ALC7-3 membranes. Therefore, PSI complexes in ALC7-3 membranes may not be organized in stable trimeric quaternary structures or are unable to form trimers after solubilization by a detergent.

The absence of PSI trimers in detergent-solubilized ALC7-3 membranes raised a possibility that the accessory subunits of PSI in general may be required for the formation of PSI trimers. To test this hypothesis we isolated photosynthetic membranes from several strains of *Synechocystis* sp. PCC6803 (Table I) and incubated them with dodecyl-β-D-maltoside (15:1 detergent to chlorophyll ratio). Resolution of monomers and trimers of PSI from these membranes revealed that trimers could be obtained from the wild type. AEK2, ADC4, AFK6, EF, DF and DE strains (Fig. 2). All these strains contain a functional *psaL* gene and the PsaL protein is present in the PS I complexes purified from them [15–19]. The proportion of trimers was drastically reduced in detergent-solubilized membranes of ADC4, DF and DE. These membranes lacked PsaD, which is required for stable assembly of many other subunits into PSI [10,11]. Chaotropic agents and detergents remove the small molecular weight subunits including PsaL from PSI of strains that lack PsaD [15]. Therefore it is likely that removal of other subunits, especially PsaL, may be responsible for the decreased yield of trimers from the membranes of PsaD-less mutants. PsaD may interact with PsaL and stabilizes its assembly in PSI. Trimers of PSI could not be obtained from the membranes of three independently generated mutant strains, ALC7-3, EL, and FL (Fig. 2). These strains lack a functional *psaL* gene and thus its product, but contain assembled PSI complexes. Therefore, PsaD, PsaE, PsaF or PsaJ may influence yield of trimers in the detergent-solubilized membranes but are not essential for the formation of PSI trimers. In contrast, the absence of PsaL led to the

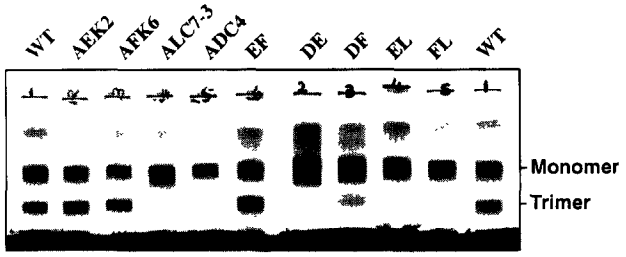

Fig. 2. Trimers of PSI cannot be obtained in PsaL-less mutants. Trimers and monomers from the membranes of different mutant strains (Table I) were resolved on sucrose-density gradients as described in Fig. 1.

lack of trimers in the detergent-solubilized membranes, indicating that PsaL is involved in trimerization of PSI.

The requirement of PsaL for the formation of PSI trimers could be due to a direct structural or an indirect regulatory role. Crystals of trimeric PSI from *Synechococcus elongatus* have been obtained and used to decipher a three-dimensional structure at 6 Å resolution [34]. Each monomer consists of a catalytic domain and a smaller domain that connects the monomers to form a trimer. It is likely that PsaL is a structural component of the trimer-forming domain. To investigate this possibility, we studied surface exposure of protein subunits in monomers and trimers of PSI by investigating their accessibility to proteolysis (Fig. 3). Monomers and trimers of PSI were incubated with thermolysin for different duration and polypeptide compositions were analyzed. PsaA-PsaB, PsaD, PsaF, PsaE, and PsaC subunits were relatively resistant to proteolysis for several hours in both monomeric and trimeric PSI. For example, ~ 90% of PsaA-PsaB remained undegraded in both forms after 4 h of protease digestion (Fig. 3, and data not shown). In contrast, PsaL was degraded in the monomeric PSI, but were resistant to proteolysis in the trimeric PSI (Fig. 3). It took approximately 4 h to completely degrade PsaL in PSI monomers. During the same time, only 15% PsaL was degraded from the trimeric PSI. Incubation of trimers with protease for 4 h at 37°C resulted in conversion of approximately 10% trimers into monomers. Therefore, the PsaL that was digested by thermolysin in trimers of PSI might be, at least partly, due to the conversion of trimers into monomers. Alternatively, degradation of PsaL might have caused dissociation of trimers. All subunits of PSI remained intact for 24 h when thermolysin was omitted during incubation (data not shown). When 0.1% SDS was added during proteolysis, all proteins in the monomeric and trimeric fractions of PSI were completely degraded by thermolysin (data not shown). Therefore, the protection of PsaL in trimers and other subunits in both forms of PSI was due to their conformation rather than due to lack of protease recognition sites. Our results demonstrate that PsaL is accessible to thermolysin in monomers and is protected in trimers of PSI, indicating that it may form at least a part of the central domain that keeps units of a trimer together.

The analysis of crystals of trimeric PSI from the cyanobacterium *Synechococcus elongatus* has revealed locations of the 4Fe–4S clusters F_x, F_A and F_B, 28 α-helices and 45 chlorophyll *a* molecules [34]. Identities of the helices are not unequivocally established. Helices *j* and *k*, which have been named arbitrarily, were presumed to be part of the PsaK subunit. They are close to the 3-fold axis in a tight bundle and therefore are proposed to be responsible for the organization of the monomers into the trimeric form [34]. We believe that the helices *j* and *k* in the trimer-forming central domain of PSI may be parts of PsaL instead of PsaK on the

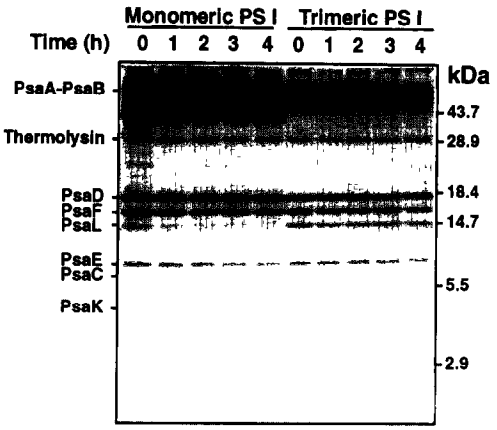

Fig. 3. Protease accessibility of PSI subunits in monomeric and trimeric complexes. The monomers and trimers of PSI were diluted to 100 μg chlorophyll/ml and incubated with thermolysin (50 μg/mg chlorophyll) and 1 mM $CaCl_2$ for 0, 1, 2, 3, 4 h. Subsequently, the reactions were terminated by addition of 25 mM EDTA and the proteins were solubilized by incubating samples with 1% SDS and 0.1% β-mercaptoethanol for 1 h at room temperature and were separated using Tricine-urea-SDS-PAGE. The proteins were stained with Coomassie blue. The experiment was repeated six times, using different periods of proteolysis. Polypeptide profiles of PSI after proteolysis in a representative experiment are shown.

basis of following reasons. First, PsaL was required for the formation of PSI trimers (Figs. 1,2). Second, PsaL was protected from proteolysis in trimers but not in monomers of PSI (Fig. 3), suggesting that PsaL is exposed on the surface of monomers that is involved in trimer-formation. Third, the ALC7-3 strain, from which trimers cannot be obtained, lacks PsaL but not PsaK [18]. PsaK was present in the PSI purified from all PsaL-less mutants. Thus the presence of PsaK was not sufficient to allow trimerization of PSI. Fourth, the suggestion that the helices *j* and *k* are parts of PsaK was based on the presence of electron density linking these helices. Hydropathy analysis of PsaL indicates the presence of a hydrophilic N-terminal region followed by three hydrophobic regions [18]. The first hydrophobic region is only approximately 13 amino acids in length and therefore may not serve as a transmembrane helix. Therefore, PsaL may contain two transmembrane helices, with a rather large N-terminal domain. The loop joining the putative transmembrane helices of PsaL contains three charged residues in *Synechocystis* sp. PCC 6803 and four charged residues in *Synechococcus elongatus*. In contrast, the putative interhelical loop in PsaK of *Synechococcus elongatus* contains five charged residues. Therefore both PsaL and PsaK are likely candidates to be responsible for the electron densities connecting helices *j* and *k*. Thus, the helices in the central domain that were identified by X-ray diffraction analysis of PSI crystals may belong to PsaL. Moreover PsaL may also connect the central domain to the catalytic domain of PSI by a rather large amino-terminal polar domain. It is likely that PsaK may have a trimer-spe-

cific, non-essential role in the organization of PSI, since it is protected in trimers but not in monomers from degradation by thermolysin (Fig. 3).

Results of the present study demonstrate that the detergent-solubilized membranes of PsaL-less mutants of *Synechocystis* sp. PCC 6803 do not contain trimers of PSI. It is, however, difficult to test if PSI in these mutants is solely in monomeric form in vivo. If the absence of trimers in vitro is also true in the cells of these mutants, it would mean that trimeric form of PSI is not essential for the function of PSI. The mutant strains of *Synechocystis* sp. PCC 6803 that lack PsaL alone [18] or in combination with other non-essential subunits of PSI (Xu, Chitnis and Chitnis, unpublished results) do not significantly differ in their photoautotrophic growth and photosynthetic activities from the wild type strain. It is possible that trimerization may play a role by modulating light-harvesting efficiency of PSI.

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REFERENCES

[1] Golbeck, J.H. (1992) Annu. Rev. Plant Physiol. Plant Mol. Biol. 43, 293–324.
[2] Chitnis, P.R. and Nelson, N. (1991) in: The Photosynthetic Apparatus: Molecular Biology and Operation (Bogorad, L. and Vasil, I.K. eds.) vol. pp. 178–224, Academic Press, San Diego.
[3] Parrett, K.G., Mehari, T., Warren, P.G. and Golbeck, J.H. (1989) Biochim. Biophys. Acta 973, 324–332.
[4] Wynn, R.M. and Malkin, R. (1988) FEBS Lett. 229, 293–297.
[5] Zilber, A.L. and Malkin, R. (1992) Plant Physiol. 99, 901–911.
[6] Lagoutte, B. and Vallon, O. (1992) Eur. J. Biochem. 205, 1175–1185.
[7] Rousseau, F., Setif, P. and Lagoutte, B. (1993) EMBO J. 12, 1755–1765.
[8] Bryant, D. (1992) in: The Photosystems: Structure, Function and Molecular Biology (Barber, J. ed.) pp. 501–549, Elsevier, Amsterdam.
[9] Oh-Oka, H., Takahashi, Y. and Matsubara, H. (1989) Plant Cell Physiol. 30, 869–875.
[10] Li, N., Zhao, J., Warren, P.V., Warden, J.T., Bryant, D.A. and Golbeck, J.H. (1991) Biochemistry 30, 7863–7872.
[11] Chitnis, P.R. and Nelson, N. (1992) Plant Physiol. 99, 239–246.
[12] Ford, R., Hefti, A. and Engel, A. (1990) EMBO J. 9, 3067–3075.
[13] Bottcher, B., Grüber, P. and Boekema, E. (1992) Biochim. Biophys. Acta 1100, 125–136.
[14] Williams, J.G.K. (1988) Methods Enzymol. 167, 766–778.
[15] Chitnis, P.R., Reilly, P.A. and Nelson, N. (1989) J. Biol. Chem. 264, 18381–18385.
[16] Chitnis, P.R., Reilly, P.A., Miedel, M.C. and Nelson, N. (1989) J. Biol. Chem. 264, 18374–18380.
[17] Chitnis, P.R., Purvis, D. and Nelson, N. (1991) J. Biol. Chem. 266, 20146–20151.
[18] Chitnis, V.P., Xu, Q., Yu, L., Golbeck, J.H., Nakamoto, H., Xie, D.-L. and Chitnis, P.R. (1993) J. Biol. Chem. 268, 11678–11684.
[19] Xu, Q., Yu, L., Chitnis, V. and Chitnis, P. (1993) J. Biol. Chem. (in press).
[20] Henry, R.L., Takemoto, L.J., Murphy, J., Gallegos, G.L. and Guikema, J.A. (1992) Plant Physiol. Biochem. 30, 357–364.
[21] Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M. and Stanier, R.Y. (1979) J. Gen. Microbiol. 111, 1–61.
[22] Arnon, D. (1949) Plant Physiol. 24, 1–14.
[23] Schagger, H. and von Jagow, G. (1987) Anal. Biochem. 166, 368–379.
[24] Takahashi, Y., Koike, H. and Katoh, S. (1982) Arch. Biochem. Biophys. 219, 209–218.
[25] Boekema, E.J., Dekker, J.P., Van Heel, M.G., Rogner, M., Saenger, W., Witt, I. and Witt, H.T. (1987) FEBS Lett. 217, 283–286.
[26] Boekema, E.J., Dekker, J.P., Rogner, M., Witt, I., Witt, H.T. and van Heel, M. (1989) Biochim. Biophys. Acta 974, 81–87.
[27] Rogner, M., Muehlenhoff, U., Boekema, E.J. and Witt, H.T. (1990) Biochim. Biophys. Acta 1015, 415–424.
[28] Rogner, M., Nixon, P.J. and Diner, B.A. (1990) J. Biol. Chem. 265, 6189–6196.
[29] Almog, O., Shoham, G., Michaeli, D. and Nechushtai, R. (1991) Proc. Natl. Acad. Sci. USA 88, 5312–5316.
[30] van der Staay, G., Boekema, E., Dekker, J. and Matthijs, H. (1993) Biochim. Biophys. Acta 1142, 189–193.
[31] Tsiotis, G., Nitschke, W., Haase, W. and Michel, H. (1993) Photosynth. Res. 35, 285–297.
[32] Kruip, J., Boekema, E.J., Bald, D., Boonstra, A.F. and Rogner, M. (1993) J. Biol. Chem. (in press).
[33] Hladik, J. and Sofrova, D. (1991) Photosynth. Res. 29, 171–175.
[34] Krauss, N., Hinrichs, W., Witt, I., Fromme, P., Pritzkow, W., Dauter, Z., Betzel, C., Wilson, K., Witt, H. and Saenger, W. (1993) Nature 361, 326–331.