Purification and Characterization of Oxygen-Evolving Photosystem II Core Complexes from the Green Alga *Chlamydomonas reinhardtii*†

Dirk Bumann and Dieter Oesterhelt*

Department of Membrane Biochemistry, Max Planck Institute for Biochemistry, am Klopferspitz 18a, 82152 Martinsried, Germany

Received February 1, 1994; Revised Manuscript Received April 25, 1994®

ABSTRACT: Oxygen-evolving photosystem II complexes were isolated from the green alga *Chlamydomonas reinhardtii* by selective solubilization of thylakoid membranes with dodecyl maltoside followed by density gradient centrifugation and anion-exchange chromatography. In the presence of $CaCl_2$ and $K_3[Fe(CN)_6]$ the complexes evolved oxygen at rates exceeding 1000 μmol (mg of chl)$^{-1}$ h^{-1}. The particles contained 40 chlorophylls *a* and had properties very similar to those of PSII isolated from higher plants. *Chlamydomonas reinhardtii* is now the first organism which can be used for both site-directed mutagenesis and detailed biochemical and biophysical characterization of oxygen-evolving photosystem II. It seems therefore to be an ideal model organism for investigation of structure–function relationships in photosynthetic oxygen evolution.

Oxygen in the earth's atmosphere is produced by oxygenic photosynthesis where water is used as an electron donor, yielding molecular oxygen as a byproduct. This water-splitting reaction is catalyzed by photosystem II (PSII),[1] which is a chlorophyll-containing multi-subunit membrane protein complex (Ghanotakis & Yocum, 1990). PSII has been isolated from various sources in different levels of complexity. Preparations of grana thylakoids contain about 250 chlorophylls per PSII and are free from photosystem I (PSI) (Dunahay et al., 1984). This material is active in oxygen evolution, but detailed biochemical and biophysical characterization is difficult due to the large number of light-harvesting chlorophylls and polypeptides. Oxygen-evolving PSII core complexes contain 35–75 chlorophylls and are mostly deficient in light-harvesting complexes (Satoh et al., 1984; Schatz & Witt, 1984; Ikeuchi et al., 1985; Tang & Satoh, 1985; Ikeuchi & Inoue, 1986; Ghanotakis & Yocum, 1987; Ohno et al., 1986; Franzen, 1987; Dekker et al., 1988; Enami et al., 1989; Haag et al., 1990; van Leeuwen et al., 1991; Adir et al., 1992). These particles are very useful for biophysical and biochemical investigations. At present oxygen-evolving particles always contain at least 33–40 chlorophylls bound to the inner core antenna polypeptides CP47 and CP43. If either of these polypeptides is removed, oxygen evolution activity is lost, but reaction centers containing 4–6 chlorophylls can be isolated which are capable of the primary photochemistry but not of the water-splitting process (Nanba & Satoh, 1987; Ghanotakis et al., 1989; Chapman et al., 1991). Besides biochemical and biophysical methods, introduction of site-directed mutations has been proven to be a very useful method for studying structure–function relationships in photosynthesis (Coleman & Youvan, 1990; Vermaas, 1993); hence an ideal model organism should allow isolation of oxygen-evolving core complexes and site-directed mutagenesis in parallel. Unfortunately no such organism exists at present. Many procedures to isolate PSII from higher plants have been described, and recently even successful crystallization of oxygen-evolving PSII has been reported (Adir et al., 1992). However, site-directed mutagenesis is not possible in higher plants at the moment. In the cyanobacterium *Synechocystis* 6803, PSII can be mutated and numerous amino acid exchanges have been made, which led to the assignment of specific functions to certain amino acids (Vermaas, 1993). However, oxygen-evolving PSII presently can be isolated from this organism only in an inhomogenous form contaminated with several other polypeptides (Burnap et al., 1989; Noren et al., 1991; Kirilovsky et al., 1992). Pure and active PSII core complexes have been isolated from other cyanobacteria, but no site-directed mutagenesis has been reported in these species (Satoh et al., 1984; Schatz & Witt, 1984; Dekker et al., 1988). Moreover, PSII of cyanobacteria has a quite different water-splitting apparatus (Vermaas, 1993) and light-harvesting system from those of PSII of higher plants (Glazer, 1989). PSII of the green alga *Chlamydomonas reinhardtii* can be mutated at specific sites, and it is very similar to PSII of higher plants with respect to the water-splitting apparatus (Mayfield et al., 1987, 1989) and the light-harvesting system (Bassi & Wollman, 1991). Moreover, numerous strains with defined deficiencies in pigment–protein complexes, which are very useful for biophysical studies, have been described (Harris, 1989). However, only PSII core complexes inactive in oxygen evolution have been isolated from this organism so far (Diner & Wollman, 1980; deVitry & Wollman, 1984; deVitry et al., 1991).

Here we report a purification procedure for homogenous, oxygen-evolving PSII core complexes from the green alga *Chlamydomonas reinhardtii*. Only one molecularly defined detergent is used, and therefore the material could be well suited for systematic crystallization experiments. Detailed characterization of these particles further supported the very close similarity of PSII from this green alga to that from higher plants. Taken together, these qualities make *Chlamy-*

† D.B. was supported by Stiftung-Stipendien-Fonds des Verbandes der Chemischen Industrie and Stiftung Volkswagenwerk by a Kekulé Fellowship.

* Author to whom correspondence should be addressed.

® Abstract published in *Advance ACS Abstracts,* July 15, 1994.

[1] Abbreviations: chl, chlorophyll; CP, chlorophyll-binding protein; cyt b_{559}, cytochrome b_{559}; DCBQ, 2,6-dichlorobenzoquinone; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; FPLC, fast protein liquid chromatography; HPLC, high-pressure liquid chromatography; kDa, kilodalton; LHC, light-harvesting complex; MES, 2-(*N*-morpholino)-ethanesulfonic acid; OEE, oxygen-evolving enhancer protein; PSI, photosystem I; PSII, photosystem II; SDS, sodium dodecyl sulfate.

0006-2960/94/0433-10906$04.50/0

Table 1: Chlorophyll Yields during Isolation of Photosystem II from *Chlamydomonas*

material	recovery of chl (%)	chl *a*/chl *b*
cells	100	2.1
membranes	95	2.1
dodecyl maltoside supernatant	70	1.9
PSII-containing band of gradient	8–12	5–6
PSII fraction from Mono Q	2.5–3.5	12–14

domonas reinhardtii an ideal model organism for studying photosynthetic oxygen evolution since genetic, biochemical, and biophysical techniques can be applied in parallel.

EXPERIMENTAL PROCEDURES

Chlamydomonas reinhardtii wild-type strain cc 125+ was grown in 20 L of liquid Tris–acetate–phosphate medium (Gorman & Levine, 1965) at 25 °C with a light intensity of 400 lx and supplied with 150 L/h sterile air. Cells were harvested at the late logarithmic phase of growth (ca. 4×10^6 cells/mL) with a flow-through centrifuge (Trennseperator TA-5-00-105, Westfalia AG Kirchheim). The following steps were carried out at 0–4 °C under green light. After the cells were washed once with buffer A (20 mM MES, 10 mM NaCl, pH 6.0), they were resuspended in 100 mL of buffer A and passed through a French press (Model FA-030, Aminco) at 28 MPa, which opened more than 98% of the cells. Addition of 400 mM sucrose before opening led to larger fragments but had no influence either on opening rate or on oxygen-evolving activity. The suspension was diluted to 260 mL with buffer A and centrifuged for 1 h at 130000*g* and 0 °C. The resulting pellet was resuspended in buffer A to a chlorophyll concentration of 1.5 mg/mL and could be stored at −70 °C after shock-freezing in liquid nitrogen for more than a month without loss of activity. The membranes were solubilized by adding 0.14 vol of a 10% dodecyl maltoside solution (detergent to chlorophyll ratio, 9:1). The sample was mixed thoroughly and centrifuged for 1 h at 110000*g* and 0 °C. Another 0.6 vol of a 10% dodecyl maltoside solution was added to the supernatant, and 4-mL aliquots were overlaid on sucrose gradients [27 mL of 20–25% sucrose, w/w, in buffer B (20 mM MES, 10 mM NaCl, pH 6.0, and 0.03% dodecyl maltoside); at the bottom of the tube we put 3 mL of 60% sucrose, w/w, to prevent sedimenting material from adhering to the tube wall]. The gradients were centrifuged for 11 h at 130000*g* and 0 °C in a TV850 vertical rotor (Kontron). The lower of the two main green bands was collected (ca. 15% of the total chlorophyll), adjusted to 130 mM NaCl, and applied to a Mono Q column (Pharmacia) attached to an FPLC system and previously equilibrated with 130 mM NaCl in buffer B. After the column was washed with 100 mL of 130 mM NaCl in buffer B, PSII was eluted with 200 mL of a gradient from 130 to 230 mM NaCl in buffer B at a flow rate of 2 mL/min. Fractions with an optical ratio (absorbance at 675 nm: absorbance at 650 nm) greater than 4.8 were collected and concentrated with Centriprep 100 (Amicon) ultrafiltration units. From 20-L cultures we obtained 4–6 mg of chlorophyll (about 3% of the chlorophyll content of the thylakoid membranes), equivalent to about 25–37 mg of PSII protein (Table 1).

Absorbance spectra were recorded with an Aminco DW2000 spectrophotometer with a 2-nm slit width. Oxygen evolution activity was measured with a Clark type electrode (Hansatech) at 25 °C in 20 mM MES, pH 5.5–7.0, or 20 mM HEPES, pH 7.6. The acceptors used were 2,6-dichlorobenzoquinone (DCBQ) and ferricyanide. Gel electrophoresis was carried out in the presence of 0.1% SDS using a separating gel with 12% acrylamide and 0.35% bis(acrylamide) as described by Randal and Hardy (1977). Samples were either applied directly (native gels; thylakoid membranes were solubilized with 2% dodecylmaltoside) or first incubated for 30 min at 37 °C in 2% SDS, 50 mM dithiothreitol, 50 mM $NaCO_3$, and 10% (w/v) sucrose (Chua, 1980) (denaturing gels). The polypeptide content of PSII particles is most often analyzed with SDS gels containing urea. However, we found that the gel system described by Randal and Hardy (1977) without urea had a superior resolution for the PSII particles from *Chlamydomonas*. Small polypeptides were separated on 16.5% acrylamide and 1% bis(acrylamide) gels using a Tricine buffer system as described by Schägger and Jagow (1987). Gels were run at 4 °C (native gels) or at ambient temperature. We used prestained molecular weight markers (Bio-Rad) or low molecular weight markers (Sigma). Gel filtration was carried out in buffer B with a Superose 6 column (Pharmacia) at a flow rate of 0.2 mL/min. The column was calibrated with standards obtained from Sigma: blue dextran (molecular mass, 2000 kDa), thyroglobulin (669 kDa), apoferritin (443 kDa), alcohol dehydrogenase (150 kDa), and carbonic anhydrase (29 kDa). Protein concentration was measured with the bicinchoninic acid method (Pierce). Chlorophyll concentration was calculated from the absorption of 80% acetone extracts as described by Porra et al. (1989). Pigment ratios were determined with normal-phase HPLC on Lichrosorb 10 (Merck) packed in a column (4 mm i.d. × 200 mm) with the eluent *n*-hexane/2-propanol/methanol, 100/0.8/0.4, at a flow rate of 2 mL/min and with a detection wavelength of 425 nm as described by Kobayashi et al. (1988). Cyt b_{559} content was measured by comparing the spectrum of a sample oxidized with ferricyanide with a spectrum of the same sample after reduction with sodium dithionite. We used the methods of both Miayazaki et al. (1989) and Cramer et al. (1986), which differ in the extinction coefficients and isosbestic wavelengths used.

RESULTS

Oxygen-evolving PSII core complexes can be isolated from higher plants with various methods. All start with an isolation of grana particles (Dunahay et al., 1984), which contain PSII and light-harvesting complex II (LHCII) but lack photosystem I (PSI) and ATPase. Comparable grana preparations from *Chlamydomonas* were also reported (Shim et al., 1990; Bassi & Wollman, 1991), but in our hands either purity or yield were unsatisfactory. Hence we developed a different isolation procedure.

We tested the detergents dodecyl maltoside, octyl glucoside, heptyl thioglucoside, C12E8, and Triton TX-100, of which only dodecyl maltoside could be used to solubilize active PSII in high yields. At a rather low detergent to chlorophyll ratio of 9 more than 95% of the oxygen-evolving activity was solubilized, but only small amounts of PSI. This selective solubilization was possible only in the absence of calcium. After ultracentrifugation to remove unsolubilized material, additional detergent was added to obtain smaller aggregates of LHC. This improved the resolution of the following density gradient centrifugation, which separates large PSII particles from smaller LHCs according to their size. Addition of 5 mM calcium to the gradient led to larger LHC aggregates, making separation from PSII impossible. At a low detergent concentration in the gradient good separation between PSII and residual PSI was achieved. PSI formed a light green band at the top of the 60% sucrose cushion. During the

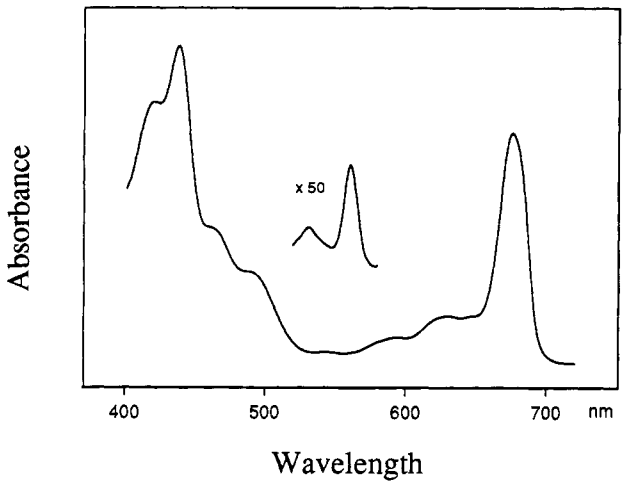

FIGURE 1: Room temperature absorption spectrum of photosystem II core complexes. The inset shows the difference spectrum between PSII core complexes oxidized with ferricyanide and those reduced with dithionite which was used used to quantitate cyt b_{559}.

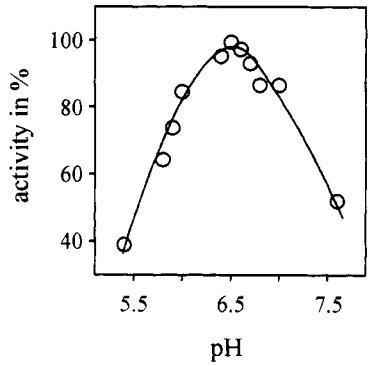

FIGURE 2: pH dependence of oxygen evolution. Two millimolar ferricyanide was added as the artificial acceptor. Maximum activity was 1000 μmol O_2 (mg of chl)$^{-1}$ h^{-1}.

following anion-exchange chromatography pure PSII eluted at about 180 mM NaCl.

Figure 1 shows the absorption spectrum at ambient temperature. The particles had a red absorption maximum at 676 nm and an optical ratio (absorbance at 676 nm/absorbance at 650 nm) larger than 4.9, indicating extensive removal of chlorophyll *b*. Light-saturated rates of oxygen evolution, measured with ferricyanide and with DCBQ as electron acceptor, were 1000–1200 and 600–700 μmol (mg of chl)$^{-1}$ h^{-1}, respectively, which are comparable to those of highly purified preparations from higher plants. The higher activity of ferricyanide compared to DCBQ indicated that the acceptor site was somewhat altered in the isolated PSII particles. In the presence of 10 μM DCMU, an inhibitor of the Q_B-binding site, oxygen-evolving activity was blocked by only 40%, further supporting a somewhat damaged acceptor site, which is a common property of highly purified PSII particles. The complexes showed optimum activity at pH 6.5, like PSII isolated from spinach (Haag et al., 1990), with 50% activity at about pH 5.6 and 7.6 (Figure 2). High rates of oxygen evolution were obtained only in the presence of calcium, with half-maximal stimulation at 6.5 mM, similar to the 8.9 mM value reported for spinach (Haag et al., 1990). We included 50 mM $CaCl_2$ in all oxygen evolution assay media to assure maximum stimulation. K_M values for the artificial acceptors ferricyanide and DCBQ measured at pH 6.0 were 128 ± 20 and 84 ± 20 μM, respectively, which are very similar to values reported for spinach (125 ± 16 and 92 ± 9 μM) (Bowlby & Yocum, 1993) at the same pH. The activity was efficiently inhibited by addition of hydroxylamine, with 50% inhibition at 130 ± 50 μM, which is somewhat lower than the value (1 mM) reported for grana membrane preparations from spinach (Ghanotakis & Babcock, 1983).

Native gel electrophoresis showed that the preparation is highly enriched in PSII and that the dominant pigment/protein complexes of the thylakoid membrane (LHC, PSI) are essentially absent (Figure 3a). This is further supported by denaturing gel electrophoresis (Figure 3b), which shows the typical five polypeptides of oxygen-evolving PSII particles with apparent molecular masses of 47 (subunit CP47), 43 (CP43), 31 (D2), 29 (D1), and 28 kDa (OEE1). ATPase and LHC polypeptides are essentially absent. Four small subunits could be resolved on high-concentration acrylamide gels (Figure 3c) which are possibly the 6.1-kDa protein and the products of genes *psb*E (cyt b_{559} α subunit), *psbM*, and *psbK* (deVitry et al., 1991). Gel filtration in the presence of dodecyl maltoside showed one homogeneous peak corresponding to a molecular mass of 230 ± 30 kDa, which shows that the particles were monodisperse (only one protein complex per mixed micelle). The protein to chlorophyll ratio was about 6. Quantitative pigment analysis with normal-phase HPLC revealed a chlorophyll *a* to pheophytin *a* ratio of 20 ± 2, indicating a chlorophyll *a* content of 40 ± 4 per PSII on the basis of the generally accepted assumption that there are two pheophytins per PSII. This value is comparable to values reported for highly purified oxygen-evolving particles from higher plants and cyanobacteria (Ghanotakis & Yocum, 1990). The chlorophyll *a* to chlorophyll *b* ratio was higher than 12, indicating again the extensive removal of light-harvesting complexes. Depending on the method of determination (Miyazaki et al., 1989; Cramer et al., 1986), the complexes contained 1.0 ± 0.15 or 1.1 ± 0.15 cyt b_{559} per 2 pheophytins (Figure 1, insert), as reported for PSII from spinach (Miyazaki et al., 1989).

DISCUSSION

Until now, PSII core complexes could be isolated from the green alga *Chlamydomonas reinhardtii* only in a form inactive in oxygen evolution, which was the major drawback for this organism to be used in investigations of photosynthetic oxygen evolution (Diner & Wollman, 1980; deVitry & Wollman, 1984; deVitry et al., 1991). Moreover, mutant strains deficient in ATPase and PSI had to be used as starting material, although for many applications a general method to isolate PSII from wild-type strains is needed. Here we present a new isolation method to obtain highly purified PSII core complexes from wild-type cells. The particles evolved oxygen at rates exceeding 1000 μmol (mg of chl)$^{-1}$ h^{-1} and had properties very similar to those obtained from higher plants. The main difference from earlier purification methods is the selective solubilization with the mild and defined detergent dodecyl maltoside, which made preservation of activity possible. Dodecyl maltoside has been shown to preserve the activity of a number of solubilized integral membrane proteins including PSII from higher plants (Franzen, 1987; Haag et al., 1990; van Leeuwen et al., 1991). Since only one detergent is used, this material might be well suited for crystallization experiments. A method employing dodecyl maltoside as the only detergent has also been described for spinach; however, only material inactive in oxygen evolution was obtained (Fotimou & Ghanotakis, 1990). All methods to isolate oxygen-evolving PSII core complexes from higher plants utilize the inhomogenous detergent Triton TX-100 to separate grana from stroma thylakoids (Dunahay et al., 1984).

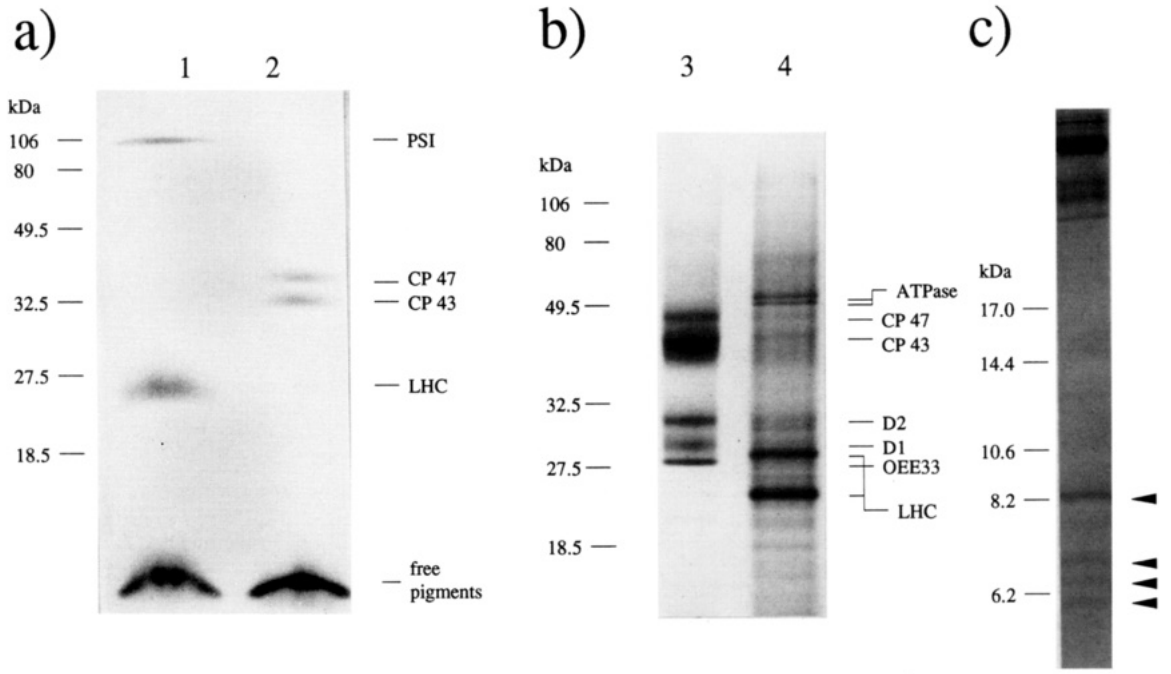

FIGURE 3: SDS–polyacrylamide gel electrophoresis of thylakoid membranes and purified photosystem II core complexes. (a) Native gel; thylakoid membranes equivalent to 2 μg of chlorophyll (lane 1) and PSII core complexes equivalent to 4 μg of chlorophyll (lane 2) were applied. (b) Denaturing gel; PSII core complexes equivalent to 0.5 μg of chlorophyll (lane 3) and thylakoid membranes equivalent to 2 μg of chlorophyll (lane 4) were applied. (c) Tricine gel with 16.5% acrylamide; PSII core complexes equivalent to 1 μg of chlorophyll were applied.

Chlamydomonas can be transformed (Boynton et al., 1988), selection markers for chloroplasts have been described (Harris et al., 1989; Takahashi et al. 1991), and deletion strains for several genes exist (Harris, 1989). Hence all prerequisites for site-directed mutagenesis of PSII are met (Boynton et al., 1992; Goodenough, 1992), and several mutations have already been introduced (Przibilla et al., 1991; Roffey et al., 1991; Erickson et al., 1992; Schrader & Johannigmeier, 1992). The isolation procedure reported here can be used to characterize the mutant PSII in detail with biophysical and biochemical methods. The ability to use a combination of genetic and biochemical methods is unique in this organism, which makes it an ideal model organism for studying structure–function relationships in photosynthetic oxygen evolution.

NOTE ADDED IN PROOF

After submission of the manuscript a method for the isolation of PSII core complexes from the cyanobacterium *Synechocystis* PCC 6803 was published (Tang & Diner, 1994). Therefore this organism is also a very good model system for the investigation of PSII.

REFERENCES

Adir, N., Okamura, M. Y., & Feher, G. (1992) in *Research in Photosynthesis, Vol. II* (Murata, N., Ed.) pp 195–198, Kluwer Academic Publishers, Dordrecht.

Bassi, R., & Wollman, F.-A. (1991) *Planta 183*, 423–433.

Bowlby, N. R., & Yocum, C. F. (1993) *Biochim. Biophys. Acta 1144*, 271–277.

Boynton, J. E., Gilham, N. W., Harris, E. H., Hosler, J. P., Johnson, A. M., Jones, A. R., Randolph-Anderson, B. L., Robertson, D., Klein, T. M., Shark, K. B., & Sanford, J. C. (1988) *Science 240*, 1534–1538.

Burnap, R., Koike, H., Sotiropoulou, G., Sherman, L. A., & Inou, Y. (1989) *Photosynth. Res. 22*, 123–130.

Chapman, D. J., Gounaris, K., & Barber, J. (1991) in *Methods in Plant Biochemistry* (Rogers, L. J., Ed.) Vol. 5, pp 171–193, Academic Press, San Diego.

Chua, N. H. (1980) in *Methods in Enzymology* (San Pietro, A., Ed.) Vol. 69, pp 434–445, Academic Press, San Diego.

Coleman, W. J., & Youvan, D. C. (1990) *Annu. Rev. Plant Physiol. Plant Mol. Biol. 19*, 333–367.

Cramer, W. A., Theg, S. M., & Widger, W. R. (1986) *Photosynth. Res. 10*, 393–403.

Dekker, J. P., Boekema, E. J., Witt, H. T., & Rögner, M. (1988) *Biochim. Biophys. Acta 936*, 307–318.

de Vitry, C., & Wollman, F.-A. (1984) *Biochim. Biophys. Acta 767*, 415–422.

de Vitry, C., Diner, B. A., & Popot, J.-L. (1991) *J. Biol. Chem. 266*, 16614–16621.

Diner, B. A., & Wollman, F.-A. (1980) *Eur. J. Biochem. 110*, 521–526.

Dunahay, T. G., Staehelin, L. A., Seibert, M., Ogilvie, P. D., & Berg, S. P. (1984) *Biochim. Biophys. Acta 764*, 179–193.

Enami, I., Kamino, K., Shen, J.-R., Satoh, K., & Katoh, S. (1989) *Biochim. Biophys. Acta 977*, 33–39.

Erickson, J. M., Whitelegge, J., Domian, A., Koo, D., Boyd, K., & Hoang, S. (1992) in *Fifth International Conference on the Cell and Molecular Biology of Chlamydomonas, Program and Abstracts Volume*, Pacific Grove, California.

Fotimou, C., & Ghanotakis, D. F. (1990) *Photosynth. Res. 25*, 141–145.

Franzen, L.-G. (1987) *Acta Chem. Scand. B41*, 103–105.

Ghanotakis, D. F., & Babcock, G. (1983) *FEBS Lett. 153*, 231–234.

Ghanotakis, D. F., & Yocum, C. F. (1987) *FEBS Lett. 197*, 244–248.

Ghanotakis, D. F., & Yocum, C. F. (1990) *Annu. Rev. Plant Physiol. Plant Mol. Biol. 41*, 255–276.

Ghanotakis, D. F., de Paula, J. C., Demetriou, D. M., Bowlby, N. R., Petersen, J., Babcock, G. T., & Yocum, C. F. (1989) *Biochim. Biophys. Acta 974*, 44–53.

Glazer, A. N. (1989) *J. Biol. Chem. 264*, 1–4.

Goodenough, U. W. (1992) *Cell 70*, 533–538.

Gorman, D. S., & Levine, R. P. (1965) *Proc. Natl. Acad. Sci. U.S.A. 54*, 1665–1669.

Haag, E., Irrgang, K.-D., Boekema, E. J., & Renger, G. (1990) *Eur. J. Biochem. 189*, 47–53.

Harris, E. (1989) *The Chlamydomonas Sourcebook*, Academic Press, New York.

Harris, E., Burkhart, B., Gillham, N., & Boynton, J. (1989) *Genetics 123*, 281–292.

Ikeuchi, M., & Inoue, Y. (1986) *Arch. Biochem. Biophys. 247*, 97–107.

Ikeuchi, M., Yuasa, M., & Inoue, Y. (1985) *FEBS Lett. 197*, 316–322.

Kirilovsky, D. L., Boussac, A. G. P., van Mieghem, F. J. E., Ducruet, J.-M. R. C., Setif, P. R., Yu, J., Vermaas, W. F. J., & Rutherford, A. W. (1992) *Biochemistry 31*, 2099–2107.

Kobayashi, M., Watanabe, T., Nakazato, M., Ikegami, I., Hiyama, T., Matsunaga, T., & Murata, N. (1988) *Biochim. Biophys. Acta 936*, 81–89.

Mayfield, S. P., Rahire, M., Frank, G., Zuber, H., & Rochaix, J.-D. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 749–753.

Mayfield, S. P., Schirmer-Rahire, M., Frank, G., Zuber, H., & Rochaix, J.-D. (1989) *Plant Mol. Biol. 12*, 683–693.

Miyazaki, A., Shina, T., Toyoshima, Y., Gounaris, K., & Barber, J. (1989) *Biochim. Biophys. Acta 975*, 142–147.

Nanba, O., & Satoh, K. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 109–112.

Noren, G. H., Boerner, R. J., & Barry, B. A. (1991) *Biochemistry 30*, 3943–3950.

Ohno, T., Satoh, K., & Katoh, S. (1986) *Biochim. Biophys. Acta 852*, 1–8.

Porra, R. J., Thompson, W. A., & Kriedemann, P. E. (1989) *Biochim. Biophys. Acta 975*, 384–394.

Przibilla, E., Heiss, S., Johannigmeier, U., & Trebst, A. (1991) *Plant Cell 3*, 169–174.

Randal, L. L., & Hardy, S. J. S. (1977) *Eur. J. Biochem. 75*, 43–53.

Roffey, R. A., Golbeck, J. H., Hille, C. R., & Sayre, R. T. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 9122–9126.

Satoh, K., Ohno, T., & Katoh, S. (1984) *FEBS Lett. 180*, 326–330.

Schägger, H., & v. Jagow, G. (1987) *Anal. Biochem. 166*, 368–379.

Schatz, G. H., & Witt, H. T. (1984) *Photobiochem. Photobiophys. 7*, 1–14.

Schrader, S., & Johannigmeier, U. (1992) *Plant Mol. Biol. 19*, 251–256.

Shim, H., Cao, J., Govindjee, & Debrunner, P. G. (1990) *Photosynth. Res. 26*, 223–228.

Takahashi, Y., Goldschmidt-Clermont, M., Soen, S.-Y., Franzen, L. - G., & Rochaix, J.-D. (1991) *EMBO J. 10*, 2033–2040.

Tang, X.-S., & Satoh, K. (1985) *FEBS Lett. 179*, 60–64.

Tang, X.-S., & Diner, B. A. (1994) *Biochemistry 33*, 4594–4603.

van Leeuwen, P. J., Nieveen, M. C., van de Meent, E. J., Dekker, J. P., & van Gorkom, H. J. (1991) *Photosynth. Res. 28*, 149–153.

Vermaas, W. (1993) *Annu. Rev. Plant Physiol. Plant Mol. Biol. 44*, 457–481.