Plant Physiol. (1985) 77, 626–629
0032-0889/85/77/0626/04/$01.00/0

Characterization of a Purified Photosystem II-Phycobilisome Particle Preparation from *Porphyridium cruentum*[1]

Received for publication September 19, 1984 and in revised form November 26, 1984

BARBARA M. CHERESKIN, JENNY D. CLEMENT-METRAL[2], AND ELISABETH GANTT*
Smithsonian Environmental Research Center, 12441 Parklawn Drive, Rockville, Maryland 20852-1773

ABSTRACT

Detergent preparations isolated from thylakoids of the red alga *Porphyridium cruentum*, in a sucrose, phosphate, citrate, magnesium chloride medium consist of phycobilisomes and possess high rates of photosystem II activity. Characterization of these particles shows that the O_2-evolving activity is stable for several hours and the pH optimum is about 6.5 to 7.2. Response of the system to light, electron donors and acceptors, and inhibitors verify that the observed activity, measured both as O_2 evolution and 2,6-dichlorophenol-indophenol reduction, is due to photosystem II. Furthermore, photosystem II is functionally coupled to the phycobilisome in this preparation since green light, absorbed by phycobilisomes of *P. cruentum*, is effective in promoting both O_2 evolution and 2,6-dichlorophenol-indophenol reduction. Photosystem II activity declines when light with wavelengths shorter than 665 nm is removed. Both 3-(3,4-dichlorophenyl)-1,1-dimethylurea and atrazine inhibit photosystem II activity in this preparation, indicating that the herbicide binding site is a component of the photosystem II-phycobilisome particle.

A PBS[3] preparation with PSII activity has been isolated from a red alga, *Porphyridium cruentum*. Phycobilisomes are the pigment protein complexes, found only in red algae and cyanobacteria, functionally analogous to Chl *a*/*b* complexes of higher plants. Energy absorbed by the PBS is primarily transferred to PSII. This leads to the assumption that the PBS is physically attached to PSII in the thylakoid membrane of red algae and cyanobacteria.

Early attempts to fractionate the photosynthetic electron transport chain resulted in loss or significant decline in the O_2 evolving activity of PSII. PSII preparations with high O_2 evolving activity were first isolated by Stewart and Bendall (20) from a cyanobacterium, *Phormidium laminosum*. Since then, similar preparations have been isolated from cyanobacteria and higher plants by others (2, 10, 11, 13, 19, 24). These workers did not attempt to retain the PBS light-harvesting complex. The specific interest in our laboratory has been to ascertain if the PBS is closely associated with the PSII components as expected from energy transfer to PSII (14–16). We had already shown that in a high ionic medium (sucrose-phosphate-citrate) it was possible to retain intact PBS and PSII activity (7). Conditions normally used for PSII preparations (2, 4, 10, 11, 13, 19, 24) result in the loss of PBS by dissociation. We recently reported the successful isolation in SPCM medium, of PIIP particles with high specific rates of PSII activity (1000–3000 μmol $O_2 \cdot mg^{-1}$ Chl $\cdot h^{-1}$) from *P. cruentum* (5). Subsequently, this same medium has been used in the isolation of PSII particles and PBS from a cyanobacterium (17).

The PIIP preparation from *P. cruentum* used in this study is deficient in PSI and has a low Chl content. By electron microscopy the preparations were shown to be discreet particles, consisting primarily of phycobilisomes (6). Thylakoid membranes were extremely rare. In this presentation we show that green light, absorbed by phycoerythrin in the PBS of *P. cruentum*, is highly effective in driving PSII in PIIP preparations. This demonstrates that PSII does not merely co-isolate with the PBS but is functionally attached. Furthermore, we show that PIIP is a true PSII particle based upon its response to light, inhibitors, and electron donors and acceptors. Inhibitor studies indicate that the herbicide binding protein is a component of the red algal photosynthetic apparatus and is also present in our PIIP preparations.

MATERIALS AND METHODS

Isolation of Phycobilisomes and PSII-Phycobilisome Particles. *Porphyridium cruentum* were grown as in Dilworth and Gantt (7) and havested after 7 d growth in the late exponential phase. The PIIP isolation was carried out in SPCM with particle solubilization using lauryl dimethylamine oxide as described in Clement-Metral and Gantt (5). PBS-(Triton)-20C and PBS-(Triton)-5C were isolated basically as in Gantt *et al.* (12) by treatment with 1% Triton X-100, followed by isolation on sucrose step gradients. The final centrifugation for 2.5 h at 254,000*g* was omitted. PBS-(Triton)-5C were isolated at 4°C.

PSII Assays. O_2 evolution was measured at 25°C with a Clark-type electrode. Light from a slide projector was filtered through 10 cm water and a Schott KG 1 heat absorbing filter. The assay volume was 3.0 ml and typically contained 2 mM FeCN, 1 mM DMBQ, and 0.67 to 1% ethanol.

The instrument was calibrated by the method of Robinson and Cooper (18). The solubility of O_2 in air-saturated SPCM was found to be about 55% of the solubility of O_2 in air-saturated water at 25°C. Therefore, values reported here are lower than those reported in Clement-Metral and Gantt (5) which were based on standardization of the electrode in air-saturated water.

DCPIP measurements were done in an Aminco DW2 spectrophotometer with side illumination from a Dolan Jenner model 170-D illuminator. The absorbance change of 560 minus 520 nm was measured with a 3 nm slit width and an extinction

[1] Supported in part by Department of Energy Contract AS05-76ER-04310 and a Smithsonian Fellowship to B. M. C.

[2] Present Address: Université de Technologie de Compiegne, B.P. 233, 60206 Compiegne Cedex.

[3] Abbreviations: PBS, phycobilisome(s); PIIP, PSII-phycobilisome; SPCM, 0.5 M sucrose, 0.5 M potassium phosphate, 0.26 M potassium citrate, 10 or 15 mM $MgCl_2$, pH 7; PBS-(Triton)-20C, phycobilisomes isolated at room temperature and solubilized with Triton X-100; PBS-(Triton)-5C, phycobilisomes isolated at 5°C and solubilized with Triton X-100; FeCN, ferricyanide; DMBQ, dimethylbenzoquinone; DCPIP, 2,6-dichlorophenol-indophenol; DPC, sym-diphenylcarbazide; I_{50}, concentration of inhibitor required for 50% inhibition.

coefficient of 5.75 $mM^{-1} \cdot cm^{-1}$. The assay was performed in 3 ml of SPCM containing 36 μM DCPIP. Chl was determined as described in Clement-Metral and Gantt (5).

Light intensity for O_2 evolution and DCPIP reduction was measured with a Gamma C9 spectral radiometer. Green light (G) was obtained with a Plexiglas filter having maximum transmittance at 528 nm and a half bandwidth of 50 nm. Red light was obtained with either a 665 or a 695 nm cut-off filter. The 665 nm cut-off filter (R_{665}) had zero transmittance below 650 nm. The 695 nm cufoff filter (R_{695}) had zero transmittance below 675 nm. Neutral density screens were used to attenuate light intensity.

RESULTS

Figure 1 shows the stability of the O_2 evolution activity in PIIP when the particles are kept at low temperatures (5°C, or −80°C). The activity initially drops within hours to about 770 μmol $O_2 \cdot mg^{-1}$ Chl $\cdot h^{-1}$ and then declines more slowly. After 25 h, 34% of the initial activity remains when the PIIP particles are kept at 5°C and 38% of the PIIP activity remains when the particle is kept at −80°C. After 3 d at −80°C, about 10% of the original activity remains. The pH optimum for the activity is about 6.5 to 7.2.

PSII activity of PIIP is further defined with various inhibitors and electron donors for PSII (Tables I and II). Table I shows that PIIP catalyzed DCPIP reduction is stimulated about 20% by

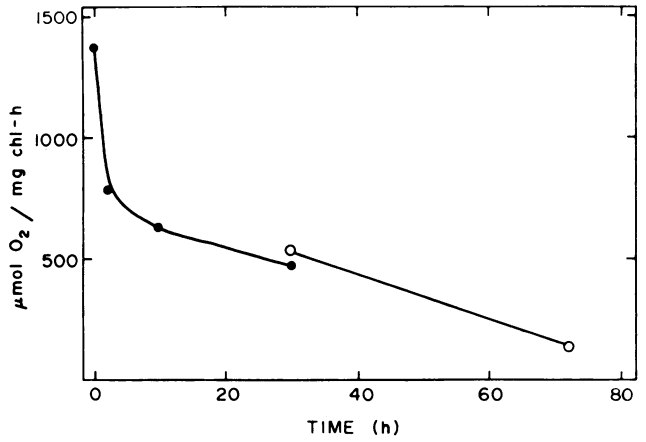

FIG. 1. Stability of O_2 evolution as a function of time at 5°C (●) or −80°C (○). O_2 evolution was measured at 25°C as described in "Materials and Methods."

Table I. *Properties of Dichlorophenol-Indophenol Reduction in PSII-Phycobilisome Particles*

DCPIP measurements were made as described in "Materials and Methods." PIIP contained 0.45 μg Chl in 3 ml SPCM; PBS-(Triton)-5C contained 0.17 μg Chl and PBS-(Triton)-20C contained trace amounts of Chl in 3 ml of 0.75 M K-phosphate (pH 6.8). DPC was added in DMSO to a final concentration of 0.3%. DCMU was added in absolute ethanol such that the final concentration was 0.67 to 1%.

	μmol DCPIP·mg⁻¹ Chl·h⁻¹
PIIP	215
PIIP + 90 μM DPC	261
PIIP + 3.3 μM DCMU	20
PBS-(Triton)-5C	44
PBS-(Triton)-5C, + 90 μM DPC	438 ± 30
PBS-(Triton)-5C, + 90 μM DPC, + 5 μM DCMU	77
PBS-(Triton)-20C	0
PBS-(Triton)-20C, + 90 μM DPC	0

Table II. *Properties of O_2 Evolving Activity in PSII-Phycobilisome Particles*

O_2 evolution was measured as described in "Materials and Methods." PBS (5C and 20C) isolated by Triton X-100 treatment contained 0.4 μg Chl in 3 ml of 0.75 M K-phosphate (pH 6.8). PIIP particles (control) contained 1 μg Chl in 3 ml of SPCM. SPCM contained 15 mM $MgCl_2$ in this experiment. All assays contained 2 mM FeCN and 1 mM DMBQ unless indicated differently. DCMU was added in absolute ethanol so that the final concentration was 0.3%. O_2 evolution was 765 ± 141 μmol $O_2 \cdot mg^{-1}$ Chl $\cdot h^{-1}$ in the control.

	% of control
PIIP	100
PIIP, + 4 mM FeCN, − DMBQ	76
PIIP, − FeCN, + 2 mM DMBQ	83
PIIP, + 2 mM NH_2OH	0
PIIP, + 1.7 μM DCMU	0
PBS-(Triton)-5C	0
PBS-(Triton)-20C	0

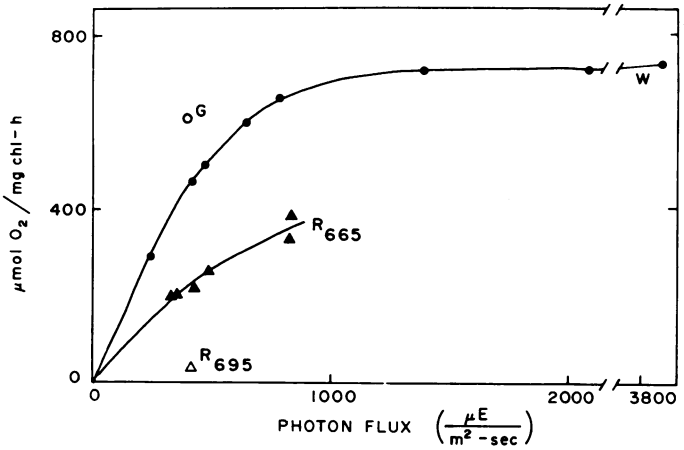

FIG. 2. O_2 evolution as a function of light intensity and light quality in PSII-phycobilisome particles. G, green light (○); W, white light (●); R_{665}, red light obtained with a 665 nm cutoff filter (▲); R_{695}, red light obtained with a 695 nm cutoff filter (△). O_2 evolution was measured as described in "Materials and Methods."

DPC and is inhibited about 90% by DCMU. Additionally, O_2 evolution is completely inhibited in PIIP particles by DCMU and NH_2OH (Table II). If either DMBQ or FeCN are omitted from the assay, rates of O_2 evolution are slightly lower. PBS isolated at 5°C (PBS-(Triton)-5C), by the conventional 1% Triton X-100 solubilization procedure of Gantt *et al.* (12), retain some DCPIP reduction activity (Table I). This activity is strongly stimulated by DPC, and inhibited about 80% by DCMU in the presence of DPC (Table I). PBS isolated at room temperature (PBS-(Triton)-20C) do not reduce DCPIP (Table I). Neither PBS-(Triton)-20C nor PBS-(Triton)-5C demonstrate O_2 evolution activity (Table II).

Figure 2 shows the effect of photon flux density and light quality on O_2 evolution activity in PIIP particles. In white light, the preparation saturates at about 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$. Half saturation occurs at about 300 $\mu E \cdot m^{-2} \cdot s^{-1}$. Green light absorbed primarily by phycoerythrin, is highly effective in supporting O_2 evolution activity. O_2 evolution activity in green light appears close to full saturation at 400 $\mu E \cdot m^{-2} \cdot s^{-1}$. At the same photon flux densities, red light, obtained either with a 665 nm filter or with a 695 nm filter, is much less effective in driving O_2 evolution in PIIP particles. Although attainment of greater photon flux densities in green and red light is limited by the light source and the filter system, O_2 evolution is consistently higher in green light

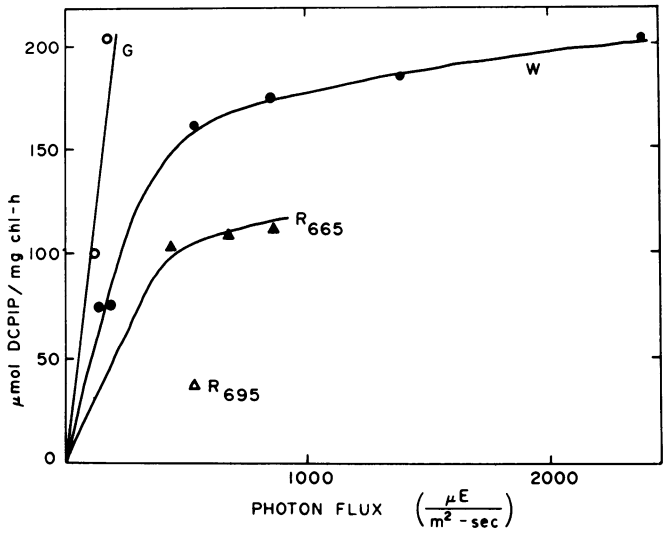

FIG. 3. Dichlorophenol-indophenol reduction in PSII-phycobilisome particles as a function of light intensity and light quality. Other conditions are described in "Materials and Methods." G, green light (○); W, white light (●); R_{665}, red light obtained with a 665 nm cutoff filter (▲); R_{695}, red light obtained with a 695 nm cutoff filter (△).

compared to white light. The results clearly show that green light is more effective. The same conclusion is drawn from the results with DCPIP reduction. Figure 3 shows an analogous experiment in which DCPIP reduction by PIIP particles is measured at different light intensities in green, white, and red light. DCPIP reduction also saturates at about 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$ with half saturation at about 300 $\mu E \cdot m^{-2} \cdot s^{-1}$. Again, green light is more effective than white light in promoting DCPIP reduction, and red light is much less effective in driving DCPIP reduction by PIIP particles.

That PIIP particles contain the PSII-associated atrazine binding protein is indicated by the inhibition of the PIIP O_2 evolving activity by atrazine (Fig. 4A). Fifty % inhibition occurs at 0.05 μM concentration and the inhibition seems to level off with 80% inhibition at about 0.4 μM, although a higher concentration of 3.3 μM produces an inhibition of 95%. When atrazine is tested on unfractionated thylakoids (Fig. 4B), higher concentrations are required for inhibition. Fifty % inhibition occurs at about 0.25 μM. The system saturates at about 4 μM atrazine with 85% inhibition, and 16 μM atrazine results in a further inhibition to 95%.

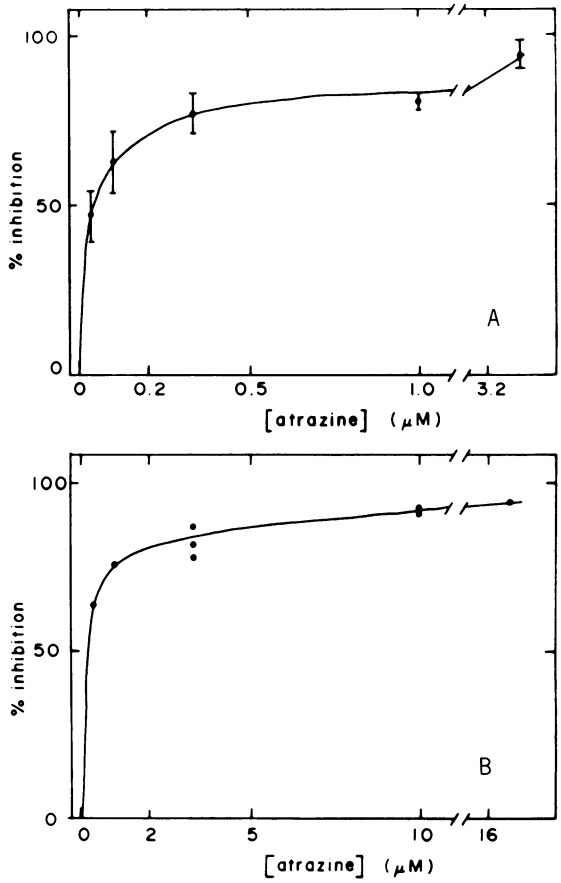

FIG. 4. Per cent inhibition of oxygen evolution activity in PSII-phycobilisome particles (A) and unfractionated membranes (B) as a function of atrazine concentration. Control activity for (A) was 805 ± 148 μmol $O_2 \cdot mg^{-1}$ Chl $\cdot h^{-1}$. Control activity for B was 288 ± 17 μmol $O_2 \cdot mg^{-1}$ Chl $\cdot h^{-1}$. O_2 evolution was measured as described in "Materials and Methods." Atrazine was added in methanol such that the final methanol concentration was 0.03 to 0.1%. Activity in the presence of atrazine was compared to appropriate controls.

DISCUSSION

The essential components of PSII are clearly present in PIIP particles and are directly linked to the PBS. This is shown by the light dependence of both O_2 evolution and DCPIP reduction (Figs. 2 and 3) and by the response of the system to inhibitors (Tables I and II; Fig. 4) and electron donors (Table I). Rates of activity based on DCPIP reduction are lower than those based on O_2 evolution. This is most likely due to the inhibition of DCPIP reduction by the high ionic strength SPCM media (22). When the PIIP preparation is diluted with 2× volume of water instead of SPCM, the rates of DCPIP reduction approximately double (data not shown).

PBS solubilized by 1% Triton X-100 retain PSII activity as measured by DCPIP reduction only when isolated at 5°C, not at 20°C (Table I). However, the absence of O_2 evolution activity and the large stimulation by DPC of DCPIP reduction indicate that the PBS(Triton)-5C particles do not contain an intact electron transport chain from water to the plastoquinone pool. Probably some component(s) on the oxidizing side of PSII have been lost. In contrast, PIIP particles evolve O_2 and are stimulated only slightly by DPC. PSII components may also be present but inactive in PBS isolated at room temperature. This would explain why PBS and PIIP preparations appear similar when analyzed by SDS-PAGE (6). Alternatively, the action of Triton X-100 at room temperature may result in a particle in which PSII is not retained with the phycobilisome.

The effectiveness of green light in driving PSII activity in PIIP particles (Figs. 2 and 3) is predictable if the PBS is functionally coupled to PSII since phycoerythrin, the green light absorber, comprises about 70% of the PBS in *P. cruentum* (7). Although the system appears close to saturation at 400 $\mu E \cdot m^{-2} \cdot s^{-1}$ of green light (Figs. 2 and 3), it is not verified since this is the maximum photon flux density available under our conditions. Red light is obviously less effective because there are fewer red light absorbing chromophores in the PIIP system.

The inhibition of PSII activity by atrazine (Fig. 4) and DCMU (Tables I and II) indicates that the herbicide binding protein is a component of our PIIP particles. I_{50} values observed in PIIP particles for atrazine are of the same order of magnitude as observed for cyanobacteria (10 nM) (1) and about 10× lower than the values reported for higher plants (21). The higher concentrations required for atrazine inhibition in unfractionated thylakoids are probably due to nonspecific binding.

The molar ratio of Chl/PBS is about 1200 in unfractionated thylakoids, when calculated by using the PBS mol wt of Dilworth and Gantt for *P. cruentum* (7). In PIIP the Chl/PBS ratio is about 60. If there are one to four PSII reaction centers per PBS (14, 16), this implies 15 to 60 Chl per PSII reaction center which is in agreement with results obtained using other approaches (3, 8, 9, 15, 23).

More than 98% of the original Chl is removed in the isolation of the PIIP particles. This decline in Chl content seems to reflect the preferential extraction of PSI Chl since PSI has been shown to be absent in these particles by low temperature fluorescence (5) and by absence of P_{700} (6).

The initial decline of this activity (Fig. 1) could be due to the partial uncoupling of electron transfer components within the particles with time. It is also possible that there are two groups of O_2 evolving centers in our preparation—some O_2 evolving centers may be stabilized by small membrane vesicles, whereas another set may depend solely on the SPCM media for retention of activity. There appeared to be no further stabilization of activity if the particles were kept at −80°C rather than 5°C. The stability of our PIIP particles is comparable to that observed by Bowes *et al.* (4) for PSII particles from *P. laminosum*. However, in contrast to these workers, we are not able to increase stability of PIIP catalyzed O_2 evolving activity by the addition of lauryl maltoside (data not shown).

In summary, our PIIP particles demonstrate high rates of O_2 evolving activity in both green and white light. This shows that the PBS are functionally coupled to the PSII activity and provides evidence for the association of PBS with PSII *in vivo* in red algae and cyanobacteria. In addition, the DCMU and atrazine sensitivity of the particle indicates that the herbicide binding protein is a component of the photosynthetic apparatus in *P. cruentum* and in the PIIP particle. Experiments in progress are expected to identify specific PSII components including P680 and RC II polypeptides.

Acknowledgments—We are obliged to Mr. John Edwards for determination of the photon flux density for Figures 2 and 3 and to Ms. Claudia Lipschultz for drawing the figures. We also appreciate Dr. A. C. Stewart's referral to the Robinson and Cooper method for calibration of the O_2 electrode.

LITERATURE CITED

1. ALLEN MM, AC TURNBURKE, EA LAGACE, KE STEINBACK 1983 Effects of photosystem II herbicides on the photosynthetic membranes of the cyanobacterium *Aphanocapsa* 6308. Plant Physiol 71: 388–392
2. BERTHOLD DA, GT BABCOCK, CF YOCUM 1981 A highly resolved, oxygen-evolving photosystem II preparation from spinach thylakoid membranes. EPR and electron transport properties. FEBS Lett 134: 231–234
3. BOWES JM, P HORTON 1982 The effect of redox potential on the kinetics of fluorescence induction in photosystem II particles from *Phormidium laminosum*. Biochim Biophys Acta 680: 127–133
4. BOWES JM, AC STEWART, DS BENDALL 1983 Purification of photosystem II particles from *Phormidium laminosum* using the detergent dodecyl-β-D-maltoside. Properties of the purified complex. Biochim Biophys Acta 725: 210–219
5. CLEMENT-METRAL JD, E GANTT 1983 Isolation of oxygen-evolving phycobilisome-photosystem II particles from *Porphyridium cruentum*. FEBS Lett 156: 185–188
6. CLEMENT-METRAL JD, E GANTT, T REDLINGER 1985 A photosystem II-phycobilisome preparation from the red alga *Porphyridium cruentum*: Oxygen evolution, ultrastructure and polypeptide resolution. Arch Biochem Biophys. In press
7. DILWORTH MF, E GANTT 1981 Phycobilisome-thylakoid topography on photosynthetically active vesicles of *Porphyridium cruentum*. Plant Physiol 67: 608–612
8. DINER B, D MAUZERALL 1973 Photosynthetic oxygen production and the size of the photosynthetic unit in a cell-free preparation from *Cyanidium caldarium*. Biochim Biophys Acta 292: 285–290
9. DINER BA, F-A WOLLMAN 1979 Functional comparison of the photosystem II center-antenna complex of a phycocyanin-less mutant of *Cyanidium caldarium* with that of *Chlorella pyrenoidosa*. Plant Physiol 63: 20–25
10. DINER BA, F-A WOLLMAN 1980 Isolation of highly active photosystem II particles from a mutant of *Chlamydomonas reinhardtii*. Eur J Biochem 110: 521–526
11. ENGLAND RR, EH EVANS 1981 A rapid method for extraction of oxygen-evolving photosystem 2 preparations from cyanobacteria. FEBS Lett 134: 175–177
12. GANTT E, CA LIPSCHULTZ, J GRABOWSKI, BK ZIMMERMAN 1979 Phycobilisomes from blue-green and red algae. Isolation criteria and dissociation characteristics. Plant Physiol 63: 615–620
13. KUWABARA T, N MURATA 1982 Inactivation of photosynthetic oxygen evolution and concomitant release of three polypeptides in the photosystem II particles of spinach chloroplasts. Plant Cell Physiol 23: 533–539
14. LEY AC 1984 Effective absorption cross-sections in *Porphyridium cruentum*. Implications for energy transfer between phycobilisomes and photosystem II reaction centers. Plant Physiol 74: 451–454
15. MIMURO M, Y FUJITA 1977 Estimation of chlorophyll *a* distribution in the photosynthetic pigment systems I and II of the blue-green alga *Anabaena variabilis*. Biochim Biophys Acta 459: 376–389
16. MIMURO M, Y FUJITA 1978 Excitation energy transfer between pigment system II units in blue-green algae. Biochim Biophys Acta 504: 406–412
17. PAKRASI HB, LA SHERMAN 1984 A highly active oxygen-evolving photosystem II preparation from the cyanobacterium *Anacystis nidulans*. Plant Physiol 74: 742–745
18. ROBINSON J, JM COOPER 1970 Method of determining oxygen concentrations in biological media, suitable for calibration of the oxygen electrode. Anal Biochem 33: 390–399
19. SCHATZ GH, HT WITT 1984 Extraction and characterization of oxygen-evolving photosystem II complexes from a thermophilic cyanobacterium *Synechococcus* spec. Photobiochem Photobiophys 7: 1–14
20. STEWART AC, DA BENDALL 1979 Preparation of an active oxygen-evolving photosystem 2 particle from a blue-green alga. FEBS Lett 107: 308–312
21. VERMAAS WFJ, KE STEINBACK, CJ ARNTZEN 1984 Characterization of chloroplast thylakoid polypeptides in the 32-KDa region: polypeptide extraction and protein phosphorylation affect binding of photosystem II-directed herbicides. Arch Biochem Biophys 231: 226–232
22. VERNON LP, ER SHAW 1969 Photoreduction of 2,6-dichlorophenolindophenol by diphenylcarbazide: a photosystem 2 reaction catalyzed by Tris-washed chloroplasts and subchloroplast fragments. Plant Physiol 44: 1645–1649
23. VERNON LP, ER SHAW, T OGAWA, D RAVEED 1971 Structure of photosystem 1 and photosystem 2 of plant chloroplasts. Photochem Photobiol 14: 343–357
24. YAMAGICHI A, S KATOH 1983 Two chlorophyll-binding subunits of the photosystem 2 reaction center complex isolated from the thermophilic cyanobacterium *Synechococcus sp.* Arch Biochem Biophys 225: 836–846