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# IDENTIFICATION OF A CHLOROPLAST MEMBRANE POLYPEPTIDE ASSOCIATED WITH THE OXIDIZING SIDE OF PHOTOSYSTEM II BY THE USE OF SELECT LOW-FLUORESCENT MUTANTS OF SCENEDESMUS

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Summary: Comparison of photochemical activities and variable fluorescence yield characteristics of whole cells and isolated chloroplast particles of low-fluorescent, photosystem II mutants of Scenedesmus obliquus to those of the wild-type showed that several strains were affected primarily on the oxidizing side of photosystem II. In strains LF-1, LF-3, and LF-5 analysis of the manganese content of isolated chloroplast membranes showed a predominant shift in the Mn/400 Chl from the wild-type value (4.3) to values near 1.5; this difference was also associated with a near total loss of cytochrome b-559 (high potential). Examination of chloroplast membrane polypeptides by gel electrophoresis revealed a decrease only in the mobility of one band in all three mutants; the apparent molecular weight was shifted from 34 kilodalton in the wild-type to 36 kilodalton in the mutants. Evidence is presented suggesting that the 34 kilodalton polypeptide of the wild-type is probably associated with the manganese requiring portion of the water-splitting apparatus of photosystem II.

One of the primary goals of current photosynthesis research has been to identify the membrane components directly involved in the water splitting reactions. It is known that approximately two-thirds of the manganese bound to the thylakoids participates in such reactions (1, 2) and that chloride ions must also be present (3). While cytochrome b-559 is believed to have a close physical association with the oxygen evolving apparatus, there is evidence against its having a direct role in electron donation to the PS-II reaction center (see Ref. 4 for a review). Current models for its function suggest other locations (5) or that it may operate on both "sides" of the PS-II reaction center and facilitate one step of the complex water splitting reactions (6). Sporatic reports have appeared suggesting the involvement of other proteinaceous factors on this side of PS-II, based on either reconstitution

ABBREVIATIONS; ASC; Na Ascorbate; Chl:chlorophyll; CP:chlorophyll-protein complex; DPIP:2,6-dichlorophenol indophenol; DPC:diphenylcarbazide; kD: kilodalton; LF:low fluorescent; MV:methyl viologen; LiDS-PAGE;lithium dodecyl-sulfate polyacrylamide gel electrophoresis; PS:photosystem; WT:wild type.

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experiments (7, 8) or on immunological grounds (9). However, no specific polypeptide of thylakoids is currently accepted as an integral component of the water splitting apparatus.

One approach to the general problem of the identification of function of membrane proteins that has proved successful has been the isolation and characterization of mutants altered in the portion of the photosynthetic system of interest (10-13). In this communication, we present the preliminary results of our characterization of three mutants of Scenedesmus obliquus blocked on the oxidizing side of PS-II, that provide strong evidence for the involvement of a membrane polypeptide (of apparent molecular weight 34 kD) in the water splitting apparatus.

## MATERIALS AND METHODS

The algal strains used in this study were the WT of Scenedesmus obliquus, strain $D_3$, and three low flourescent, photosynthetic deficient mutants (LF-1, LF-3, LF-5) obtained from the WT by x-ray irradiation. Maintenance and growth of the algal strains were done by methods previously described (13). The low flourescent mutants having a specific deletion affecting the oxidizing side of PS-II were selected by a combination of the fluorescence method for mutant detection and replicate plating techniques (14).

Chloroplast membrane fragments for gel electrophoresis studies were prepared by the methods described earlier (15). Except for minor modifications the procedures for solubilizatin of chloroplast membranes and the lithium dodecylsulfate-PAGE (LiDS-PAGE) were identical to those of Delepelaire and Chua (16). For molecular weight calibration a 10% acrylamide gel, rather than the 7.5-15% gradient gel, was prepared and samples run with standardized protein markers, (Sigma Chemical Co.). Following electrophoresis the gel slab was photographed to register the position of the chlorophyll-protein complexes, and subsequently fixed and stained in a solution of 0.2% Coomassie brilliant blue (R-250), acetic acid (7%) and methanol (25%). Photographic records of the slabs were made following destaining.

Except for minor changes, the photochemical activities of chloroplast particles prepared from the various algal strains were measured as previously described (13, 14). Manganese analysis of isolated chloroplast memebranes was made either by atomic absorption (Instrumentations Laboratories, Inc.) using the digestion procedure suggested in part E of (18) or by neutron activation analysis. Thylakoid membranes were prepared as previously indicated with precautions taken to exclude extraneous manganese. The final membrane pellet was suspended in deionized water and chlorophyll concentrations determined. For neutron activation analysis aliquots were dispensed into polyethylene vials; these, and appropriate manganese standards, were heated to dryness, sealed and the contents activated by exposure to thermal neutrons in a TRIGA nuclear reactor. Manganese concentrations were determined by comparing the corrected gamma ray photon peak size at 846 KeV (arising from decay of $^{56}Mn$ as measured with a lithium-drifted germanium semiconductor detector system) to a standard curve which was linear over the range examined.

Variable yield fluorescence was measured by previously described methods (17). The PS-II specific electron donor system of ascorbate and hydroquinone

(20) was added to the whole cell suspension to a final concentration of 2mM HQ and 3mM ASC when indicated.

## RESULTS AND DISCUSSION

The mutants used for this study were initially selected on the basis of their inability to grow photoautotrophically on a minimal medium, for their retention of a low fluorescence yield and for their retention of the in vivo photosystem I-dependent reduction of carbon dioxide (photoreduction) (14, 23). Several lines of evidence indicate that they are blocked on the oxidizing side of PS-II but possess an otherwise intact and functional photosynthetic system. The data in Table 1 show that whole cells or chloroplast fragments of these mutants cannot use water as an electron source, but when alternate donors for PS-II (DPC) or PS-I ($H_2$ or $DCIPH_2$) are provided, they possess reaction rates comparable to those of the WT controls. The variable yield fluorescence patterns (Figure 1) reveal the loss of the variable yield component and a strong 650 or 712 light off-transient (the corresponding

TABLE 1. RATES OF PHOTOSYNTHESIS AND VARIOUS PHOTOCHEMICAL REACTIONS OF WHOLE CELLS AND CHLOROPLAST PARTICLES OF WT AND SELECT LOW FLUORESCENT, PS-II MUTANTS OF SCENEDESMUS.

| Strain Designation | Photosynthesis[a] | DPIP-MV[b] | $H_2O$-DPIP[c] | DPC-DPIP[c] | Mn/400 Chl |
|---|---|---|---|---|---|
| Wild-Type | 53 | 330 | 63 | 21 | 4.3 ± 0.7 (n=3) |
| LF-1 | Trace | 350 | 0 | 26 | 1.6 ± 0.2 (n=3) |
| LF-2 | Trace | 310 | 0 | 29 | 1.4 ± 0.1 (n=2) |
| LF-3 | Trace | 310 | 0 | 21 | 1.5 |

[a]Rates of photosynthesis given as μmoles $O_2$ $mg^{-1}$Chl $h^{-1}$ as determined at saturating levels of redlight with wavelengths greater than 620 nm. Temperature = 28°C.

[b]PS-I catalyzed reduction of methyl viologen measured polargraphically as μmoles $O_2$ consumed $mg^{-1}$ Chl $h^{-1}$ at 25°C.

[c]Rates presented as μmoles DPIP reduced mg $^{-1}$Chl $h^{-1}$ at saturating light intensities and at wavelengths greater than 620 nm. For the DPC-DPIP determination, Tris buffer extracted (20) WT chloroplast membranes were used for the control.

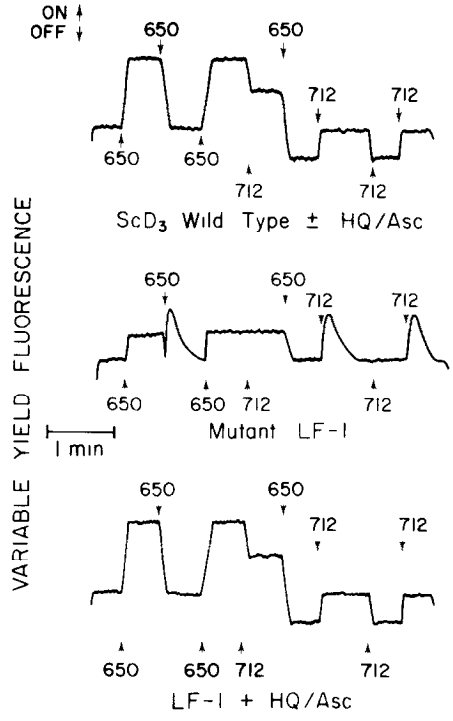


Figure 1. Comparison of the influence of PS-II and PS-I wavelengths of light on the variable yield fluorescence of whole cells of WT and LF-1 with and without the addition of HQ/ASC. 3 µl of cells were resuspended in 3 ml of 0.05M $KH_2PO_4$ - $K_2HOP_4$ buffer, pH = 6.5, just before measurements were made.

responses in LF-3 and LF-5 were virtually identical). However, when ASC-HQ is provided as a PS-II electron donor system (20), the fluorescence patterns noted for the WT is recovered. This donor system does not alter the pattern noted for the WT. The post illumination transient seen in LF-1 is probably related to conformational changes in chloroplast structure and appears to be amplified in PS-II deficient materials.

Analysis of the manganese content of isolated thylakoids of the WT indicates a Mn/400 Chl of between 4 and 5 (Table 1), values that are in agreement with the work of others for Scenedesmus (22). All of the mutants show a decrease in this ratio by approximately two-thirds. Interestingly, it has been shown that two-thirds of the membrane bound manganese is active in the oxygen evolving reactions (1, 2). The manganese content of whole cells of LF-1 is not significantly different from that of the WT (data not shown) indicating that there is not a general impairment in uptake ability.

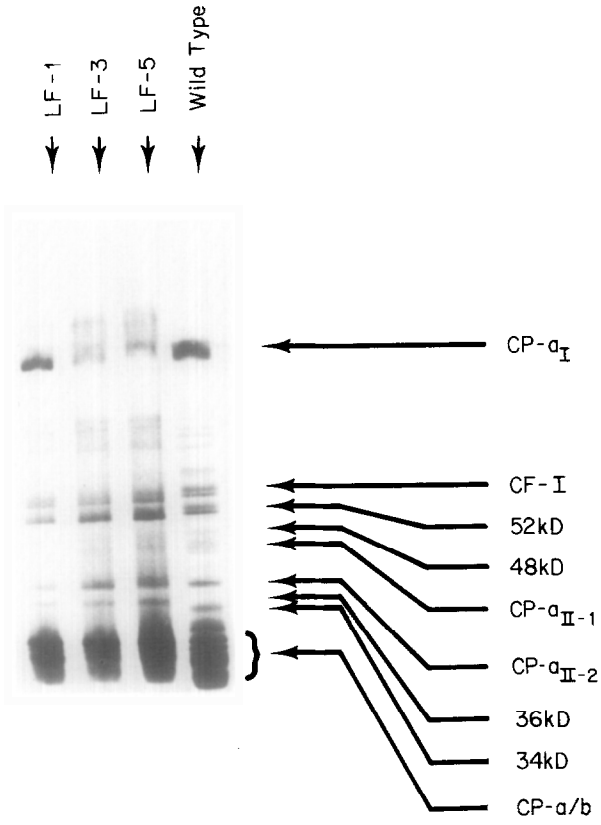


Figure 2. Chlorophyll-protein complexes and thylakoid membranes polypeptides of WT and mutants LF-1, LF-3, and LF-5. Analysis was made by LiDS-PAGE at 4°C. Samples containing 15 µg of chlorophyll were added for each algal sample. Gels were stained with Coomassie brilliant blue R250.

We have used the LiDS-PAGE method described by Delepelaire and Chua (16) to analyze and compare the composition of the thylakoid membranes of the WT and the mutants. Examination of the stained gel (Figure 2) reveals that a polypeptide of apparent molecular weight 34 kD in WT has an altered mobility (to apparent molecular weight 36 kD) in the mutants; this is the only difference clearly discernable in the patterns. Prior to fixing and staining of the gels, we noted the same CP complexes in Scenedesmus as were seen in Chlamydomonas (16). However, we have not observed a chlorophyll containing zone in the region of the 34 kD band as did Delepelaire and Chua (16) on occasion.

Since LF-1 is deficient in cytochrome b-559 H.P. but retains a near normal content of total b-type cytochromes (b-559 + b-563) (23), it does not appear likely that the 34 kD band is associated with this protein. This interpretation is supported by the finding that the removal of manganese from the chloroplast membrane results in the conversion of the high potential to lower potential forms (24, 25) without a decrease in total cytochrome b-559. Additionally, published values for the molecular weights of the oligomeric and monomeric forms of cytochrome b-559 (26, 27) appear unrelated to the 34 kD membrane component.

Recent findings on membrane proteins of mitochondria and chloroplasts show that certain specific ones are synthesized and inserted into the membrane in a precursor form and are subsequently processed by removal of a small polypeptide chain to yield a physiologically active membrane component (28, 29). Our observation of an apparently identical decrease in electrophoretic mobility of a chloroplast membrane polypeptide in several independently isolated phenotypes (LF-1, LF-3, and LF-5) suggests that a precursor-product relationship exists between the 36 kD protein observed in the mutants and the 34 kD protein of the WT Scenedesmus. Changes in apparent molecular weight of chloroplast membrane polypeptides similar to those noted here have been reported to occur during development of the photosynthetic process in Zea maize (29) and Spirodela oligorrhiza (30); however, no attempt was made to assign physiological roles for these proteins.

Experiments are in progress to characterize the 34 kD protein of Scenedesmus to establish in more detail its relationship to the 36 kD protein and its possible homology to polypeptides of similar molecular weights in other photosynthetic organisms.

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