Short Communication

Synthesis of a Photosystem I Polypeptide of 15 Kilodaltons in Isolated Etiochloroplasts of Wheat

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ABSTRACT

Etiochloroplasts isolated from greening wheat (Triticum aestivum L. cv Norin 61) seedlings synthesized a membrane polypeptide of 15 kilodaltons. One-dimensional peptide mapping with Staphylococcus aureus V8 protease revealed that the 15 kilodalton polypeptide is the subunit 5 of photosystem I reaction center complex.

The PSI reaction center purified from several higher plants consists of 6 to 7 subunit polypeptides (2, 3, 8, 11–13). The largest subunit of 65 to 70 kD, an apoprotein of CPI (P700-Chl a-protein), was synthesized in isolated chloroplasts from several plants (5–7, 18), and the gene for this polypeptide has been found on ctDNA (17). However, there has been little information on the synthesis of the small subunit polypeptides of 10 to 25 kD in isolated chloroplasts.

The etiochloroplasts isolated from wheat seedlings in early phases of the greening actively synthesized the membrane polypeptide of 15 kD, but its function was not determined (15). In the present study, this 15 kD polypeptide is compared with a small subunit of PSI with the aid of one-dimensional peptide mapping.

MATERIALS AND METHODS

Plant Material and Culture Conditions. Wheat (Triticum aestivum L. cv Norin 61) was cultured in two ways as previously described (15). For the preparation of chloroplasts used for the protein synthesis experiment, 8-d-old seedlings grown in the dark were greened under continuous light (3000 lux with white fluorescent tubes) for 18 h. The PSI reaction center was purified following the method of Nelson (13) with slight modifications. The CF1-deficient membrane fraction was resuspended in 0.4 M sucrose, 10 mM NaCl, 5 mM MgCl2, and 10 mM Tricine-KOH (pH 8.3) to give a 1.1 mg/ml concentration of Chl. Digitonin was added to the supernatant to a concentration of 1% and incubated for 1 h, then centrifuged at 30,000g for 10 min. NaCl and digitonin were added to the supernatant to give concentrations of 0.1 M and 1.5%, respectively. After an overnight incubation, the mixture was spun at 20,000g for 3 h. The pellet was resuspended in 1.25 ml of 0.4 M sucrose, 10 mM NaCl, 10 mM HEPES-NaOH (pH 8.3), and 2% Triton X-100 to give a 0.72 mg/ml concentration of Chl. After an overnight incubation, the mixture was applied on a DEAE cellulose (Whatmann DE-23) column (0.75 x 6 cm). The column was washed with a solution containing 50 mM Tris-HCl (pH 8.3) and 0.2% Triton X-100.

An arrow indicates the unidentified 15 kD polypeptide.
V8 aureus clear: methanol (1:1, v/v) carried films purified centrifugation in used previously PAGE for Triton collected in w/v) adjoining above above X-OMAT 706 PSI Polyacrylamide Gel Electrophoresis and Determination of Radioactivity. PAGE was carried out as previously described (15) except for the following modifications. SDS in the gel system previously used (15) was replaced by LDS, and electrophoresis was carried out at 4°C.

Determination of radioactivity on the dried gels was carried out by autoradiography (15) or fluorography using Kodak X-OMAT R films. For the fluorography, the gels were gently shaken in 4 volumes of Enlightening (New England Nuclear) methanol (1:1, v/v) for 30 min, then dried and exposed to x-ray films at -70°C.

Identification of Polypeptides by Partial Proteolysis. Identification of polypeptides by partial proteolysis with Staphylococcus aureus V8 protease (Miles Lab.) was carried out according to

FIG. 2. PSI reaction center polypeptides of wheat. PSI reaction center was purified according to the method of Nelson (13) and electrophoresed in the buffer system of Laemmi (10). A part of the left gel is enlarged in adjoining half lane. Subunit polypeptides are denoted 1 to 6 in the order of decreasing mol wt.

X-100, and the reaction center complex was eluted with the above buffer containing 0.13 M NaCl. Fractions of green band were collected and applied on sucrose density gradient (5–25%, w/v) in a solution containing 20 mM Tris-HCl (pH 8.0) and 0.2% Triton X-100, then centrifuged at 100,000g for 15 h. After a centrifugation the lower green band was collected and used as a purified PSI reaction center.

RESULTS AND DISCUSSION

The membrane polypeptides synthesized in the isolated etiochloroplasts of wheat are shown in Figure 1. The arrow indicates the unidentified polypeptide of 15 kD. The etiochloroplasts isolated in early phases of the greening synthesized this polypeptide, but matured chloroplasts did not (15). This polypeptide was not removed from the membrane by washing with 2 mM NaBr, though CF1 was removed by this washing (data not shown). This indicated that the 15 kD polypeptide was not the constituent of CF1 complex.

The PSI reaction center of wheat consisted of at least 6 polypeptides of 66, 20, 18, 18, 15, and 10 kD (Fig. 2). They are denoted 1 to 6 in the order of decreasing mol wt. The electrophoretic mobilities of the polypeptides of 3 and 4 are close to each other, and they are clearly distinguishable in the enlarged photograph (Fig. 2, right lane). The polypeptide pattern of the PSI reaction center shown in Figure 2 is similar to those of other higher plants (3, 8, 11, 12, 16, 17).

One-dimensional peptide mappings of [14C]methionine labeled 15 kD polypeptides and the subunit 5 of PSI reaction center are shown in Figure 3. The former polypeptide was detected by fluorography while the latter by staining with Coomassie brilliant blue. Partial proteolysis of the subunit 5 of PSI reaction center produced three or four fragments, and three of them corresponded to those of [14C]methionine labeled polypeptide as marked in Figure 3. These results indicate that the radioactive polypeptide of 15 kD is the subunit 5 of PSI reaction center.

Nechushtai et al. (12) studied the site of synthesis of the PSI reaction center polypeptides by in vivo inhibition experiments on Spirodea, and they obtained the result that the subunit V of PSI reaction center appeared to be of chloroplast origin. The
subunit 5 in our PSI reaction center preparation probably corresponds to the subunit V obtained by Nechushtai et al. (12) judging from its electrophoretic profile in Tris-glycine-SDS gels (11–13).

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LITERATURE CITED