Short Communication

Chlorophyll-Protein Complexes of a Photosystem II Mutant of Maize

EVIDENCE THAT CHLOROPHYLL-PROTEIN a-2 AND A CHLOROPHYLL-PROTEIN COMPLEX DERIVED FROM A PHOTOSYSTEM I ANTENNAE SYSTEM COMIGRATE ON POLYACRYLAMIDE GELS

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ABSTRACT

Use of the octyl β-d-glucopyranoside solubilization procedure of Camm and Green (1980 Plant Physiol 66: 428-432) reveals that thylakoid membranes of a photosystem (PS) II-deficient maize (Zea mays L.) mutant lack two chlorophyll protein (CP) complexes associated with PSII, i.e., CPa-1 and CPa-2. In contrast, when lithium dodecyl sulfate is used to solubilize the membranes of the mutant prior to electrophoretic separation, a CP complex is observed which has a mobility similar to that of CPa-2. Comparison of spectral characteristics and polypeptide composition of the green bands in this region taken from samples of the mutant, normal sibling control plants and from PSII preparations indicate that the CP complex observed in the mutant represents a portion of a light-harvesting complex of PSII (Mullet et al. 1980 Plant Physiol 65: 814-822). The green band observed in normal maize samples can contain both the CPa-2 complex as well as the CP complex derived from the PSII antennae system.

Recent improvements in methods involving PAGE of chloroplast membranes have allowed the detection of pigment-protein complexes believed to represent the reaction center and an immediate antennae system of PSII (1, 5, 7, 9, 10, 19). The complexes each contain a single polypeptide, β-carotene, and Chl a (but not Chl b) (5, 7, 14, 19). In maize, Zea mays L., the apoproteins of these complexes have apparent molecular masses of 49 and 45 kD (15). Various terminology has been used for these two CP complexes. In this report, we will use CPa-1 for the complex whose apoprotein = 49 kD, and CPa-2 for the complex whose apoprotein = 45 kD.

Previously, we have shown that the nuclear recessive, mutant of maize, designated hcf-3, is lacking the entire PSII complex (15). We have now discovered in CP complexes what was observed that during short term LDS-PAGE, thylakoid samples from hcf-3 possessed a CP complex with a mobility similar to that of CPa-2 (preliminary data was presented in 11). In this report, we show that the complex observed in hcf-3 is not related to any component of PSII, but is derived from an antennae system of PSI, which has been termed LHC I (8, 17). Also, the data suggest that, in samples of normal maize thylakoids, the green band observed in this region can be due to the presence of both CPa-2 and the complex related to LHC I.

MATERIALS AND METHODS

Techniques for growth and detection of maize (Zea mays L.) seedlings homozygous for the hcf-3 trait have been published (16). Preparation of thylakoid membranes for electrophoresis was as in Metz and Miles (15). The method of Kuwabara and Murata (12) was used to obtain OEPSII, except that a Triton X-100 to Chl ratio of 22.5:1 (rather than 25:1) was employed. The lower detergent concentration resulted in a small contamination with PSI but was necessary to obtain an OEPSII pellet from maize.

CP complexes were separated using LDS-PAGE (7). A 4.5% acrylamide stacking gel and 10% acrylamide separating gel were employed. Octyl glucoside treatment of isolated thylakoid membranes was as described in Camm and Green (6). For solubilization with LDS, samples containing 1 mg Chl/ml were made to 1% LDS and 30 mM DTE just prior to electrophoresis at 4°C (12.5 m amp constant current) (gel dimensions = 1.5 mm x 14 cm) for 3 h. Spectra of excised portions of the gel were obtained with an Aminco DW-2. For polypeptide analysis, gel slices were soaked in 30 mM DTE for 15 min then placed in the sample wells of a second gel and overlaid with 0.1 ml of 0.4 M NH4HCO3, 0.5 mM phenylmethylsulfonil fluoride, 30 mM DTE, 7% glycerol, 1% LDS. The slices were allowed to incubate at room temperature for 3 h prior to electrophoresis at 4°C at 3 w constant power. Other samples were prepared as before except that they were heated (4 min at 70°C) prior to loading on the gel. The second gel, which had a linear 10 to 15% acrylamide gradient, was prepared and stained as in Metz and Miles (15).

RESULTS AND DISCUSSION

Solubilization of thylakoid membranes with the nonionic detergent octyl glucoside prior to PAGE stabilizes the CP complexes.

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4 Abbreviations: CP, Chl-protein; hcf, high chlorophyll fluorescence; LDS, lithium dodecyl sulfate; LHC, light-harvesting Chl-protein complex; OEPSII, oxygen evolving PSII preparation; octyl glucoside, octyl-β-d-glucopyranoside.
of PSII (5, 6). This is especially important for CPA-1, which is more labile than CPA-2 in most systems. An added feature of Camm and Green’s procedure is that it depletes the sample of PSI components (they are removed by a high speed centrifugation step). Figure 1A shows a typical separation of CP complexes when thylakoids from hcf-3 and its normal sibling control plants are treated with octyl glucoside prior to LDS-PAGE at 4°C. All of the samples contain the CP complexes of the light-harvesting system normally associated with PSI, i.e. CPA/b and CPA/b*.

As expected, thylakoids of hcf-3 are missing the CPA-1 and CPA-2 bands. These bands are present in both normal maize and OEPSII samples.

Most analyses of CP complexes have been made with samples solubilized with SDS or LDS (e.g. 18). Figure 1B shows the CP pattern obtained when thylakoids and OEPSII are solubilized with 1% LDS, instead of octyl glucoside, prior to electrophoresis. Both hcf-3 and the normal maize samples have the CP complex associated with the PSI reaction center (CPI). The OEPSII shows a small amount of this band. The CPA/b and CPA/b* bands are present in all of the samples. As noted in Bricker et al. (4), the OEPSII is enriched in CPA/b*. CP complexes with mobilities between CPA/b* and CPA/b have been described many times, and have been suggested to be associated with the reaction center of PSI. In the gel shown, all of the samples (including the PSII-deficient mutant) have a CP band in this region.

The in situ absorption spectra of these bands are shown in Figure 2. The band from the OEPSII shows the characteristics classically assigned to the CPA-2 complex (7, 9, 19). It has a peak at 671.5 nm and there is no shoulder near 650 nm, indicating the lack of Chl b. The spectrum of the band from hcf-3 has a peak at 674.5 nm and clearly has a contribution from Chl b. For the gel shown in Figure 1B, the green band of the normal control plants has a peak at 673 nm and a slight shoulder at 650 nm. The spectral characteristics of the bands from hcf-3 (i.e. no PSI) and the OEPSII (i.e. no PSI) are very consistent. In contrast, we have found that the peak position (in the red region) of the band from normal thylakoids (with both PSI and PSI) can vary from 671.5 to 674 nm and that as the peak shifts to the red the Chl b shoulder becomes more pronounced. This suggests that the green band in normal samples may be due to two comigrating species. These species are individually present in the samples from hcf-3 and the OEPSII.

Figure 3 shows results of an analysis of the polypeptide content of the gel slices used to obtain the spectra of Figure 2. Both the apoproteins of the CP complexes as well as other polypeptides which comigrate with the complexes in the short gel are revealed by this procedure. All three gel slice samples (lanes 2, 4, and 6) show stained protein in the 38 to 40 kD region, which corresponds to the mobility of the green bands excised from the first gel. These lanes also contain polypeptides whose mobilities are significantly altered, suggesting that they were present in the first gel as noncovalent complexes. In the sample from the OEPSII (lane 6), such a protein is present with an apparent size of 45 kD. This band represents the apoprotein of the CPA-2 complex (e.g. 7, 19). A 45 kD protein is also present in the OEPSII sample (lane 5) as well as normal thylakoids (lane 1) and the CP band from normal thylakoids (lane 2). Both the thylakoids of hcf-3 (lane 3) and the CP complex of hcf-3 (lane 4) are missing the 45 kD band.

Polypeptides with altered mobilities are also present in the CP complex from hcf-3. These polypeptides, with apparent molecular masses of 25 to 26 kD, are present in thylakoids of hcf-3 as well as thylakoids of normal maize and the CP band taken from normal thylakoids (lanes 1 and 2). The resolution in this portion of the gel varies, but generally three bands can be distinguished. These polypeptides are lacking in the OEPSII and in the CP band taken from OEPSII (lanes 5 and 6). A pigment-protein complex, which acts as an antennae system for PSI has recently been isolated and characterized from peas (8, 17). The isolated complex, which is called LHC I, has an absorption peak near 674 nm, contains Chl b (a/b ratio = 3.7), and contains a group of polypeptides in the 19 to 24 kD region as well as a 10 kD polypeptide. It is likely that the complex observed in hcf-3 is derived from LHC I. The lack of a 10 kD polypeptide in the gel indicates that the CP complex does not represent the entire LHC I. We have labeled this CP band LHC I* to indicate it is a portion of the in vivo complex. CP complexes, having mobilities less than that of CPI, have been described in both Chlamydomonas (21) and in higher plants (2), and have been shown to contain polypeptides believed to be associated with the LHC I. These preparations have a more extensive polypeptide composition than is indicated for the CP complex labeled LHC I* in Figure

![Figure 1](https://academic.oup.com/plphys/article/75/1/238/6079523) Chlorophyll-protein complexes of thylakoid membranes from the PSI-deficient maize mutant (hcf-3), the normal sibling plants (WT), and maize OEPSII preparation as resolved by LDS-PAGE at 4°C. Samples were solubilized prior to electrophoresis with: (A) 30 mM octyl glucoside or (B) 1% LDS. The gel was not stained and the dark bands represent green CP complexes except for the free pigment band (FP) running at the electrophoretic front.
which would account for their different mobility.

Both the spectrum and the polypeptide composition of the CP
band from normal maize samples indicate that LHC I*, as well
as CPa-2, is present in the gel. We have noted that the two
complexes are not always equally represented. Various factors
such as temperature, power, and length of the electrophoretic
procedure appear to affect the ratio. Although we cannot predict
with consistency which complex will be favored, the composition
of the green band can be determined by examination of the
absorption spectrum. If the LHC I* is present, the peak will not
be at 671.5 nm but will be shifted further to the red. The presence
of Chl b in CP complexes from this portion of gels has been
noted by others (e.g., 3, 20) and has generally been ascribed to
contamination by CP a/b. We suggest the source of the Chl b
may also be due to small amounts of the LHC I* complex.

The presence of a CP complex in LDS-PAGE patterns which
migrates in the position of a complex previously assigned to PSII
has been listed as evidence that thylakoids of hcf-3 contain the
reaction center of PSII (13). We show in this report that the CP
band in hcf-3 is not related to CP complexes which have been
assigned to PSII. We also suggest that this complex represents
a portion of the antennae system of PSI. Finally, we have noted
that the LHC I* CP complex can also be present in samples from
normal maize thylakoids and its possible presence should be
considered when analyzing this region of the gels.

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