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The chlorophyll *ab* complex, CP29, is associated with the Photosystem II reaction centre core

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Photosystem II particles from spinach prepared by an octylglucoside method (Ghanotakis et al. (1987) Biochim. Biophys. Acta 891, 15–21) totally lack the antenna chlorophyll-protein complex LHC II, but do contain CP47, CP43 and a third chlorophyll-protein complex, as shown by non-denaturing SDS electrophoresis. This latter core-associated complex is shown to be CP29 on the basis of: (i) the apparent molecular weight of the holocomplex; (ii) the presence of Chl *b* as well as Chl *a* in excised gel slices of the complex; and (iii) the cross reaction of the apoproteins with polyclonal antibodies to barley CP29 and with monoclonal antibodies to the N-terminus of the apoprotein of pea CP29. The PS II particles are also enriched in a 23 kDa polypeptide of unknown function.

Introduction

More than half the chlorophyll (Chl) in a higher plant belongs to the Chl *a/b* complexes which function as light-harvesting antennae in both photosystems. The diversity and complexity of these Chl proteins are just beginning to be appreciated [1–3]. In PS II alone, there are at least three separate *a/b* complexes: the major one, LHC II, which is usually seen in both oligomeric ((CP II*) and monomeric (CP II) forms on mildly denaturing gels; a less prominent complex CP29, which can be resolved in the presence of octyl glucoside; and a minor complex, CP24 [4]. A fourth complex, CP27, may be a subset of LHC II [5].

CP29 is distinguished from LHC II in several ways. It has a Chl *a/b* ratio of 3–5 compared to 1.2 [3,4]. In some species, e.g., barley, its apoprotein has a higher apparent molecular weight than the LHC II polypeptides [6,7]. The proteins also have different proteolytic fragmentation patterns [8]. That CP29 is a distinct complex is supported by the deduced amino acid sequence of the cloned gene: it is only slightly more related to the LHC II (Cab) multigene family than it is to the LHC I antenna polypeptides (Pichersky, E. et al., unpublished results). At the physiological level, CP29 and LHC II appear to differ in their mobility in the thylakoid membrane. LHC II is generally considered to be at least partially mobile and to redistribute between grana and stroma lamellae (appressed and non-appressed regions of thylakoids) in response to physiological conditions (for references, see Ref. 1). Immuno-electron microscopy shows CP29 localized in the granal regions under all conditions [9]. On a number of grounds it has been suggested that CP29 is a core antenna, i.e., it is closely associated with the photochemically active core complex of PS II [1,10,11].

If these models are correct, and LHC II is only transiently attached or not attached at all to PS II cores [11], it should be possible to devise a method for purifying PS II particles which have no LHC II but still retain CP29. We have found that enriched O_2-evolving PS II reaction centre complexes prepared by the method of Ghanotakis et al. [12,13] are indeed such particles. We show here that they contain the Chl *a/b* complex CP29 as well as the two Chl *a* antenna complexes CP47 and CP43, and have little or no LHC II. The identity of CP29 was confirmed using antibodies specific for this complex.

Materials and Methods

Preparation of PS II-active fractions

Washed thylakoid membranes were prepared from market spinach as previously described [14]. PS II membranes ('BBY particles') were prepared by the method

Abbreviations: LHC II, light-harvesting antenna chlorophyll-protein complex; CP II, LHC II monomer; Chl, chlorophyll; BBY and G&Y particles are called after authors of Refs. 15 and 13, respectively; Mes, 4-morpholineethanesulphonic acid.

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of Berthold et al. [15] as modified by Dunahay et al. [16]. Enriched O_2-evolving PS II core particles ('G&Y particles') were prepared from PS II membranes with octyl glucoside in the presence of high salt by the method of Ghanotakis et al. [12]. In some experiments, the membranes were washed with 1 M NaCl, 10 mM Mes buffer (pH 6.5) to remove the extrinsic 23 kDa polypeptide of the watersplitting complex [17].

Chlorophyll assays

Chl was measured in fractions in 80% acetone using the equations of Lichtenthaler and Wellburn [18]. The hydroxylamine method of Ogawa and Shibata [19] was used for the determination of Chl *a/b* ratio.

SDS gel electrophoresis and immunoblotting

Undenatured chlorophyll-protein complexes were resolved by a modification of previous procedures [6,8]. Samples were washed in 2 mM Tris-maleate (pH 8.0) and pelleted. The pellet was suspended in 300 mM octyl glucoside (Sigma) in the same buffer to give a detergent/Chl ratio of 30 and stirred for 2–4 min at 4° C. It was then diluted with 2–3 volumes of 40% glycerol in Tris-maleate (pH 8.0), loaded on a 10% acrylamide gel topped by a 2 cm long stacking gel. Electrophoresis was at 20 mA for 4–5 h in the dark at 4° C.

Electrophoresis of denatured samples was by modifications of [8]; (a) on a 7.5–15% acrylamide gradient gel with 1.3 M Tris-HCl (pH 9.8) (Fig. 4) or (b) a 10% polyacrylamide gel, 0.375 M Tris-HCl (pH 8.8), with 4 M urea in both stacking and resolving gels. Samples for electrophoresis were solubilized in 65 mM Tris-HCl (pH 6.8), 20 mM dithiothreitol, 10% ethylene glycol and 2% SDS for 30 min at 42° C before use.

Polypeptides were located on the nitrocellulose after electrotransfer but before immunoblotting by temporary staining with Poinceau S (Chlamydomonas Newsletter No. 14, Mike Adams, Biology Department, ECSU, Willimantic, CT 06226, U.S.A.).

Polypeptides were identified by immunoblotting as previously described [6,20]. The polyclonal antiserum was raised against barley CP29 which had been purified by electrophoresis on at least two different concentrations of acrylamide to remove contaminating polypeptides. The antiserum was purified by pre-adsorption with an *Escherichia coli*-lambda gt11 wt lysate, then by pre-adsorption with purified tomato LHC II isolated by the Triton-cation precipitation method of Burke et al. [21]. This LHC II preparation does not contain any CP29, and serves to remove all antibody molecules reacting with epitopes common to CP29 and LHC II.

Polypeptides of CP29 were also identified by reaction against the monoclonal antibody MLH-2 [22] used at a 1:500 dilution.

Results

Photosystem II membranes were first prepared from washed spinach thylakoids [16], then treated with 70 mM octyl glucoside in the presence of high concentrations of NaCl and sucrose [12]. The resulting enriched PS II particles were highly active, reducing DCPIP at a rate of 500–850 μmol per mg per h with water as a donor. Since DCPIP is not a particularly efficient acceptor for purified PS II [23], this is probably an underestimate of the true activity.

Fig. 1 shows a 'green gel' of PS II membranes ('BBY particles') and enriched O_2-evolving PS II particles lacking LHC II (G&Y particles') dissociated with 300 mM octylglucoside and separated by SDS-PAGE under mildly denaturing conditions. In the BBY particles, most of the LHC II is preserved in its oligomeric form CP II*. This allows the separation of CP29 from CP II, the LHC II monomer. This method of solubilization also gives improved resolution of the recently-described CP24 [4]. The G&Y particles (right-hand lane) have only three Chl-protein bands: two which are identifiable as the two Chl *a* complexes CP47 and CP43, and a band which migrates at the position of CP29. These particles contained no detectable LHC II in either the monomer or oligomer form.

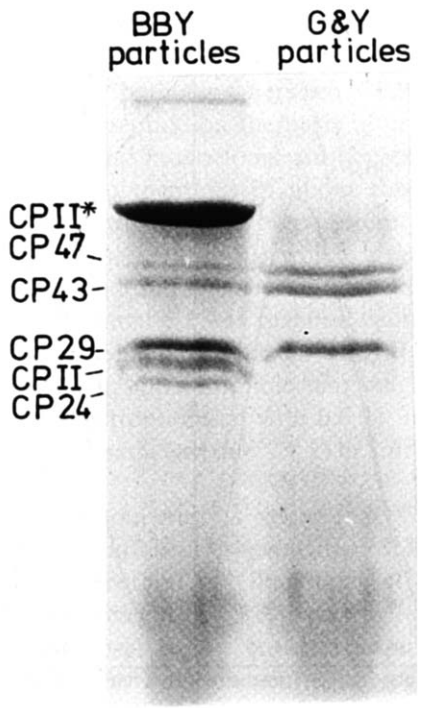

Fig. 1. Unstained gel ('green gel') showing Chl-protein complexes of BBY particles and G&Y particles. Particles were washed in 2 mM Tris-maleate (pH 8.0), solubilized with 300 mM octylglucoside at a detergent/Chl ratio of 30/1, diluted with 3 volumes of 40% glycerol in the same buffer, and separated on a 10% polyacrylamide gel (unstained). Each lane contains 25 μg chl.

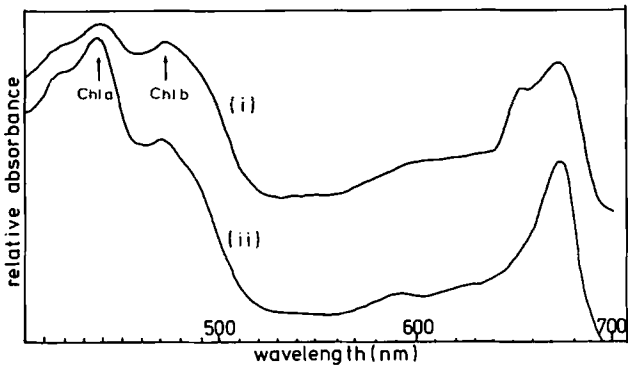

Fig. 2. Visible spectra of gel slices from a gel similar to that in Fig. 1. (i) CP II*; (ii) CP29.

The absorption spectra of CP29 and CP II* in gel slices are compared in Fig. 2. They are identical to published spectra of purified complexes from spinach and other higher plants [24]. The spectrum of CP29 shows a distinct Chl *b* peak in the region of 475 nm and a shoulder around 650 nm. In order to verify the presence of Chl *b* in CP29, complexes in gel slices were extracted with methanol and the spectra compared before and after the addition of hydroxylamine (Fig. 3). Hydroxylamine converts Chl *b* to its oxime but has no effect on Chl *a* [19]; this is a more sensitive method of determining a high Chl *a*/*b* ratio than the method of Arnon [25]. A chl *a*/*b* ratio of 5 was calculated for

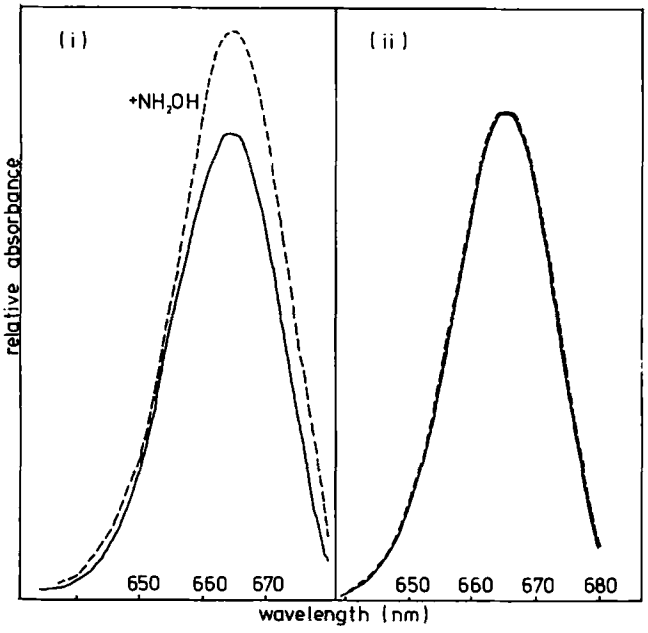

Fig. 3. Spectral changes demonstrating the formation of the Chl *b* oxime in pigments extracted from excised green bands from gels. (i) Methanolic extract of CP29 from G&Y particles; (ii) combined methanol extracts of the Chl *a*-containing complexes CP47 (CPa-1) and CP43 (CPa-2). Solid line: methanolic extract alone; dashed line: spectrum 5 min after the addition of 40% hydroxylamine. Curves are corrected for dilution.

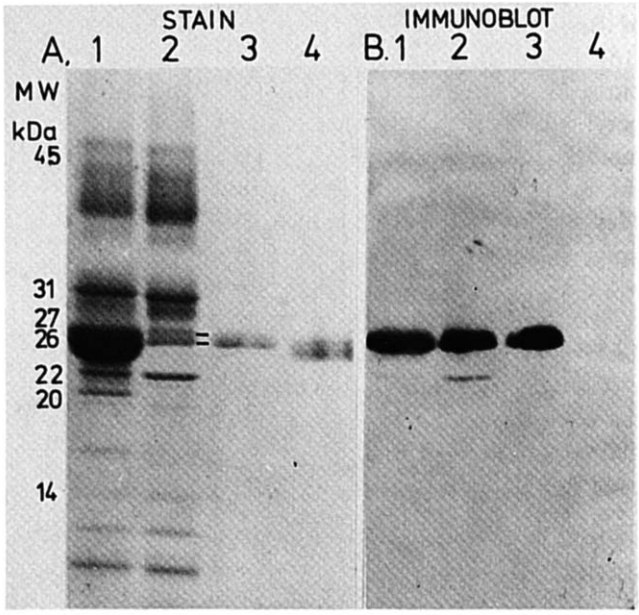

Fig. 4. Polypeptides of PS II particles and Chl-protein complexes, separated by electrophoresis on a 7.5–15% polyacrylamide gradient containing 1.3 M Tris-HCl (pH 9.8), 0.5% SDS. Lane 1, NaCl-washed BBY particles; lane 2, G&Y particles; lane 3, CP29 purified by electrophoresis on 10% and 7.5% gels; lane 4, CP II* excised from a gel similar to that in Fig. 1. (A) Coomassie stain. BBY particles, 10 μg Chl; G&Y particles 5 μg Chl. The 26 and 27 kDa polypeptides of CP 29 are marked. (B) Immunoblot against purified polyclonal anti-CP29. BBY particles, 5 μg Chl; G&Y particles, 2.5 μg Chl.

CP29 prepared in this way, compared to 3–4 for CP29 from *Acetabularia* assayed by the same method [26].

The polypeptides of BBY and G&Y particles are compared with those of purified CP29 and CP II* in Fig. 4. The G&Y's have two closely spaced bands at the same position as CP29 apoprotein(s) with apparent molecular weights of 26 and 27 kDa in this system. Unfortunately, electrophoretic mobility cannot be used to distinguish LHC II and CP29 polypeptides in spinach, as they run very close together on all gel systems we have tried [8]. To be sure that these were the CP29 polypeptides, samples were immunoblotted with antiserum raised against highly purified barley CP29 [6,20]. In barley, CP29 polypeptides are well-separated from those of LHC II on a denaturing gel. In addition, the antiserum was exhaustively pre-adsorbed with purified LHC II to remove antibodies reacting with those epitopes that are common to all Chl *a*/*b* proteins (see Materials and Methods). That this procedure was effective is shown on the two rightmost lanes of Fig. 4. The antiserum reacted only with polypeptides of purified CP29 and did not react with those of CP II* (LHC II). Panel B shows that G&Y's are enriched in polypeptides recognized by this purified antibody, confirming that CP29 is indeed part of this PS II particle.

The polypeptides of BBY and G&Y particles were also immunoblotted with the monoclonal antibody MLH-2, which is specific for the N-terminal end of pea

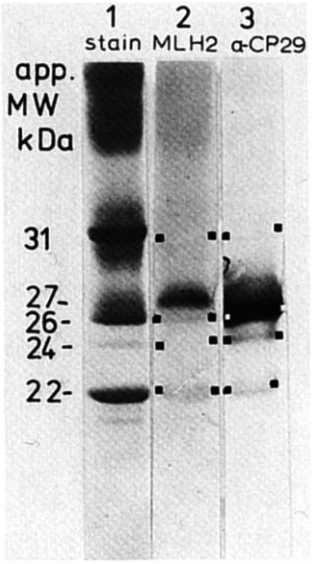

Fig. 5. Polypeptides in spinach G&Y particles recognized by polyclonal antibodies (anti-CP29, purified), or by monoclonal antibodies (MLH-2). 10% polyacrylamide gel containing 4 M urea and 0.375 M Tris-HCl (pH 8.8). Lane 1: Coomassie-stain; lane 2: immunoblot with monoclonal antibody MLH-2; lane 3: Immunoblot with purified polyclonal anti-CP29 (see Materials and Methods). Squares show positions of major protein bands as located by staining with Poinceau S. The weakly immunostained band at 24 kDa in lane 3 does not appear in all preparations. It is not part of LHC II, since purified anti-CP29 does not recognize LHC II polypeptides, as shown in Fig. 4.

CP29 [22] (Fig. 5). This antibody reacts more strongly with the upper of the two CP29 polypeptides. On the same gel, the polyclonal antibody reacts equally with both bands.

In addition to the CP29 band, the polyclonal anti-CP29 and the monoclonal MLH-2 both show a weak positive reaction with a polypeptide of 23 kDa (Figs. 4 and 5). This weakly immunopositive polypeptide is always detected in spinach G&Y particles by anti-29, and a faint band at this position is sometimes detectable in BBY particles (Fig. 4). It is not detected in the green CP29 holocomplex excised from gels (Fig. 4). It is not likely to be a proteolytic product of the CP29 apoproteins, since the relative amount of the 23 kDa band is unchanged when protease inhibitors are included in isolation buffers, but a specific proteolytic cleavage cannot be ruled out because there is at least one reported chloroplast protease insensitive to all known inhibitors [27]. The identity of this band is currently under investigation (Camm, E.L. and Green, B.R., unpublished results).

Discussion

Our analyses show that CP29 does indeed contain Chl *b*, as previously reported by a number of labs [3,4,7,8]. In our enriched PS II preparation, two polypeptides (apparent molecular masses, 26 and 27 kDa), which are immunostained with purified anti-29, are definitely associated with a green complex which contains both Chl *a* and *b*. In contrast, Ghanotakis et al. [12] reported the presence of a 28 kDa polypeptide which was associated only with Chl *a* after removal from G&Y particles by gel filtration in the presence of dodecyl maltoside.

Both polypeptides from our preparation react with a highly purified polyclonal antiserum which is specific for CP29, and which does not recognize LHC II. They are therefore not simply residual LHC II polypeptides. In addition, both of these CP29 polypeptides are recognized by the monoclonal antibody MLH-2 which is specific for the N-terminal end of the CP29 apoprotein. However, one of the bands reacts more strongly than the other. In tomato, there is only one gene for CP29 apoprotein, but the single 30 kDa precursor synthesized in vitro from the cloned gene is processed into two polypeptides of about 26 kDa when taken up by intact tomato chloroplasts (Pichersky, E. et al., unpublished results). This suggests that the two polypeptides isolated from the CP29 holocomplex could be processing variants of one polypeptide (as shown in Ref. 29). Since MLH-2 is specific for the N-terminal end of the CP29 polypeptide, a second processing site just a few amino acids downstream could significantly decrease antibody binding. However, it is conceivable that one of the two polypeptides in CP29 might only bind Chl *a*, consistent with the suggestion of Ghanotakis et al. [12].

In the current work, we have also detected some potential sequence homology between CP29 and a 23 kDa intrinsic polypeptide on the basis of cross reactivity to polyclonal and monoclonal antibodies. There are several intrinsic polypeptides of about this molecular weight, identified in different preparations by various workers (e.g., Refs. 17 and 30) which are difficult to compare without additional criteria. With this in mind, we simply note that the PS II reaction centre complex preparations of Ghanotakis et al. [12,13] contain a polypeptide with an apparent molecular weight of about 22 kDa (when electrophoresed with 6.5 M urea), which may well be the same as our 23 kDa intrinsic polypeptide.

In conclusion, our results show that CP29 is an integral part of enriched O_2-evolving PS II particles prepared by the method of Ghanotakis et al. [12,13], and support the concept of CP29 as part of an internal antenna in PS II [1,10,11]. In our current working model of PS II, the reaction centre polypeptides D1, D2, cytochrome *b*-559 and the psbI 4.8 kDa gene product [28] are in close association with CP47, CP43 and the 33 kDa extrinsic polypeptide to form the inner core, with an outer layer consisting of CP29 and several lower molecular weight polypeptides as proposed by Murata and Miyao [30]. It appears that the association of LHC

II with the above-mentioned components is only peripheral. This is consistent with the current thought that some LHC II units are so separable as to be able to migrate into unappressed regions of the thylakoid, and also with the suggestion that loosely associated LHC II units frequently fracture with the PFs fracture face rather than the EFs face [11].

References

1 Staehelin, L.A. (1986) in: Encyclopaedia of Plant Physiology, Vol. 19, New Series, (Staehelin, L.A. and Arntzen, C.J., eds.), pp. 1–84, Springer-Verlag, Berlin.
2 Green, B.R. (1988) Photosynth. Res. 15, 3–32.
3 Chitnis, P.R. and Thornber, J.P. (1988) Photosynth. Res. 16, 41–64.
4 Dunahay, T.G. and Staehelin, L.A. (1986) Plant Physiol. 80, 429–434.
5 Dunahay, T.G., Schuster, G. and Staehelin, L.A. (1987) FEBS Lett. 215, 25–30.
6 White, M.J. and Green, B.R. (1987) Eur. J. Biochem. 163, 545–551.
7 Machold, O., Simpson, D.J. and Møller, B.L. (1979) Carlsberg Res. Commun. 44, 235–254.
8 Camm, E.L. and Green, B.R. (1980) Plant Physiol. 66, 428–432.
9 Dunahay, T.G. and Staehelin, L.A. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. II, pp. 701–704, Martinus Nijhoff, Dordrecht.
10 Camm, E.L. and Green, B.R. (1983) J. Cell. Biochem. 23, 171–179.
11 Greene, B.A., Staehelin, L.A. and Melis, A. (1988) Plant Physiol. 87, 365–70.
12 Ghanotakis, D.F., Demetriou, D.M. and Yocum, C.F. (1987) Biochim. Biophys. Acta 891, 15–21.
13 Ghanotakis, D.F. and Yocum, C.F. (1986) FEBS Lett. 197, 244–248.
14 Camm, E.L., Green, B.R., Allred, D.R. and Staehelin, L.A. (1987) Photosynth. Res. 13, 69–80.
15 Berthold, D.A., Babcock, G.T. and Yocum, C.F. (1981) FEBS Lett. 134, 231–234.
16 Goodman-Dunahay, T.G., Staehelin, L.A., Seibert, M., Ogilvie, P.D. and Berg, S.P. (1984) Biochim. Biophys. Acta 764, 179–193.
17 Ljungberg, U., Åkerlund, H.-E. and Andersson, B. (1986) Eur. J. Biochem. 158, 477–482.
18 Lichtenthaler, H.K. and Wellburn, A.R. (1983) Biochem. Soc. Trans. 11, 591–592.
19 Ogawa, T. and Shibata, K. (1965) Photochem. Photobiol. 4, 193–200.
20 White, M.J. and Green, B.R. (1987) Eur. J. Biochem. 165, 531–535.
21 Burke, J.J., Ditto, C.L. and Arntzen, C.J. (1978) Arch. Biochem. Biophys. 187, 252–264.
22 Darr, S.C., Somerville, S.C. and Arntzen, C.J. (1986) J. Cell Biol. 103, 733–740.
23 Ogilvie, P.D., Reschl, L.B. and Berg, S.P. (1983) Arch. Biochem. Biophys. 220, 451–458.
24 Camm, E.L. and Green, B.R. (1981) Plant Physiol. 67, 1061–1063.
25 Arnon, D.L. (1949) Plant Physiol. 24, 1–15.
26 Green, B.R., Camm, E.L. and Van Houten, J. (1982) Biochim. Biophys. Acta 681, 248–255.
27 Kirwin, P.M., Elderfield, P.D. and Robinson, C. (1987) J. Biol. Chem. 262, 16386–90.
28 Ikeuchi, M. and Inoue, Y. (1988) FEBS Lett. 241, 99–104.
29 Kohorn, B.D. and Tobin, E.M. (1986) Plant Physiol. 82, 1172–1174.
30 Murata, N. and Miyao, M. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. I, pp. 453–462, Martinus Nijhoff, Dordrecht.