Characterization of Photosystem II Antenna Complexes Separated by Non-Denaturing Isoelectric Focusing

Grzegorz Jackowski[a] and Stefan Jansson[b]

[a] Department of Plant Physiology, Adam Mickiewicz University, Al. Niepodleglosci 14, 61–713 Poznañ, Poland
[b] Department of Plant Physiology, University of Umea, 901 87 Umea, Sweden

Z. Naturforsch. **53c,** 841–848 (1998); received February 9/April 27, 1998

Chlorophyll *a/b*, Protein, Light-Harvesting, Polypeptide, Subcomplex, Trimer

CP26, CP29 and three different LHC II subcomplexes have been purified from a carnation photosystem II (PSII) preparation using non-denaturing isoelectric focusing in a vertical polyacrylamide slab gel. The identity of the fractions was established by absorption spectroscopy, SDS-PAGE and immunoblotting. CP26 comprised a single apoprotein of 26.6 kDa and CP29 contained two apoproteins of 28.8 and 28.5 kDa. LHC II subcomplex A consisted of Lhcb1 homotrimers, and subcomplexes B and C consisted of Lhcb1/Lhcb2 and Lhcb1/Lhcb3 heterotrimers, respectively. We discuss the data in relation to the organization of the PS II antenna *in vivo*.

Introduction

The light-harvesting chlorophyll *a*/*b*-binding protein complexes of photosystem II (PSII) are a family of pigment-proteins which account for the majority of PSII chl and protein. They bind chl *a* and *b*, and the xanthophylls lutein, neoxanthin, violaxanthin, antheraxanthin and zeaxanthin (Jansson, 1994). The main light-harvesting complex of PS II, LHC II, binds more than 50% of the chl in the thylakoid membrane (Chitnis and Thornber, 1988). However, LHC II is not restricted to PSII; it can also serve as an antenna for PSI. Apart from acting as a light-harvesting antenna, this complex is involved in thylakoid adhesion (Armond *et al.*, 1976), and in the acclimation of the photosynthetic apparatus to short-term and prolonged changes in the light regime. (Allen *et al.*, 1981; Larsson *et al.,* 1987). Besides LHC II, three minor chl *a/b*-proteins have been found in PSII particles, named CP29, CP26 and CP24 according to their apparent molecular weights during non-denaturing electrophoresis (for review, see Jansson, 1994). It is generally accepted that these complexes function as intermediates in transfer of excitation energy from LHC II to PSII reaction centers although it is suggested that CP29, CP26 and CP24 may also serve as sites of thermal deactivation of excess excitation energy within PSII (Horton *et al.*, 1996). The related PsbS (CP22) protein has recently been found to bind chl *a* and *b* (Funk *et al.*, 1994), although its precise function is unknown.

The light-harvesting chlorophyll *a/b*-protein complexes of photosystem II are encoded by the nuclear *Lhcb* genes (Jansson *et al.,* 1992). *Lhcb1* and *Lhcb2* encode the two major LHC II proteins (Lhcb1 and Lhcb2, respectively) while *Lhcb3* encodes a minor LHC II protein (Lhcb3) that seems to be confined to PSII, and lacks the phosphorylation site at the N-terminus found in the major proteins. CP29, CP26 and CP24 apoproteins are encoded by the *Lhcb4*, *Lhcb5* and *Lhcb6* genes, respectively and are sometimes referred to as Lhcb4, Lhcb5 and Lhcb6. For the biochemist, the group of Lhcb proteins present a number of analytical problems. They are all of similar size, but variations in gel mobility between species and gel systems and microheterogeneities within each species makes unambiguous identification without the use of antibodies tedious. Their immunological

Abbreviations: CBB G-250, Coomassie Brilliant Blue G-250; chl, chlorophyll; CP29, CP26 and CP24, chlorophyll *a/b* proteins 29, 26 and 24, respectively; DM, *n*-dodecyl-β-D-maltoside; IEF, isoelectric focusing; LDS, lithium dodecyl sulfate; LHC II, the main light-harvesting chlorophyll *a/b*-protein complex of photosystem II; nd, non-denaturing; OG, octyl-β-D-glucopyranoside; pI, isoelectric point; PSII, photosystem II.

Reprint requests to Dr. G. Jackowski.
Fax: +4861 8523615.
E-mail: grzegorz@rose.man.poznan.pl

0939–5075/98/0900–0841 $ 06.00

identification is not straightforward either, since the high level of homology between the proteins reduces the specificity of antibodies raised against even highly purified Lhcb proteins. They are also very hydrophobic, so purification of the proteins requires solubilisation from the thylakoid membrane using relatively high concentrations of detergents. Since the pigment-protein interactions are often weaker than the protein-protein interactions, the detergent-purified proteins do not retain their original pigment content. Nonionic, glucosidic detergents are the best alternatives in this respect, but their use leads, nevertheless, to significant and irreproducible pigment loss. Thus, the pigment composition has not been unequivocally determined for any of the Lhcb proteins, (as reviewed in Jansson, 1994). As an example, Lhcb4 has been variously reported to bind from 4 to 13–14 chl (*a+b*) molecules per monomer and the corresponding range of values reported for CP26 is between 5 and 13–14 chl (*a+b*)/monomer (Barbato *et al.*, 1989; Dainese and Bassi, 1991; Henryrsson *et al.*, 1989; Lee and Thornber, 1995).

CP29, CP26 and CP24 seem to be monomeric proteins but LHC II is trimeric. Over the years, the term "monomeric LHC II" has been frequently used, but it seems likely that "monomeric LHC II" corresponds either to CP29, CP26 and CP24, to LHC II trimers that have dissociated into monomers during preparation or to monomers that have not yet assembled into trimers (Dreyfuss and Thornber, 1994). Today, there is little doubt that in the mature, green thylakoid, Lhcb1 and Lhcb2 occur exclusively as trimers which in turn can assemble into more highly-ordered structures (Jackowski and Kluck, 1994; Peter and Thornber, 1991). For Lhcb3, the situation is not clear. Although sometimes assumed to be a monomeric protein, there is evidence that it may occur in trimers with Lhcb1 or Lhcb2 (summarized in Jansson, 1994). A lot of different LHC II trimers can be resolved by IEF (Bassi *et al.*, 1988; Jackowski, 1996; Jackowski and Przymusinski, 1995) but the biochemical differences between the different trimers are still not understood. One obvious source of variation is that the trimers may be composed of different permutations of Lhcb1 and Lhcb2 subunits. However, phosphorylation of individual apoproteins, and minor variations within populations of Lhcb1 and Lhcb2, which are encoded by multi-gene families (Jansson *et al.*, 1992) are likely to add to the heterogeneity. The number of different trimers that can be obtained varies between species and gel systems, but is normally between three and six.

The present study was undertaken to clarify some of these ambiguities. A procedure allowing mild, efficient and rapid purification of carnation CP29 and CP26 suitable for reliable analysis is described. These fractions, and three LHC II trimeric fractions, were analyzed using monospecific antibodies yielding new biochemical data on the PSII LHC proteins. We demonstrate in this report that each of the three different LHC II trimers has a unique polypeptide composition.

Materials and methods

Plant material

The studies were carried out using carnation (*Dianthus caryophyllus* L. cvs Rosalie, Galil) leaves obtained from a local supplier. We had previously found that carnation leaves provide a suitable starting material for studies concerning polypeptide composition of LHC II trimeric complexes (Jackowski and Przymusinski, 1995) as LHC II trimers from carnation can be easily prepared and resolved with better performance than in other species, e g spinach (Spangfort and Andersson, 1989) and barley (Jackowski, 1996). With the present study, a more detailed knowledge of the PSII LHC proteins of carnation has been achieved.

Isolation and fractionation of PSII complexes

PSII complexes were isolated as described by Enami *et al.* (1989), except that 300 mM KCl was used instead of 40 mM $MgCl_2$ during n-HTG-dependent solubilization of PSII particles (Jackowski and Kluck, 1994). The PSII complexes were recovered from the supernatant by centrifugation at 300 000 × *g* for 30 min, the resulting pellet was washed extensively with 2 mM EDTA, pH 7.00 and stored frozen in 10% glycerol.

For separation of fractions, the PSII complex-containing preparation was treated with DM and resolved by vertical slab gel ndIEF as described previously (Jackowski and Przymusinski, 1995) except that 0.47% DM was included in the gel. The green fractions of PSII complex were electroe-

luted from the gel using Model 422 Electroeluter (BioRad, Italy) equipped with membrane caps of cutoff 12–15kDa.

Sucrose gradient ultracentrifugation

LHC II subcomplexes A-C were electroeluted from ndIEF gels using 20 mM Tris/maleate, pH 7.0, as an elution buffer. The samples were adjusted to 0.375 mg chl/ml and LDS, OG and DM were added to yield the final concentrations of 0.3%, 0.6% and 0.3%, respectively. The samples were incubated on ice for 30 min. and spun in 0.2–0.8M sucrose gradients as described previously (Jackowski, 1996).

SDS-PAGE and immunoblotting

SDS-PAGE of all samples was performed using the buffer system of Laemmli (1970). Immunoblot analyses were performed as outlined earlier (Jansson *et al.*, 1997).

Spectroscopic analyses

Steady-state absorption spectra were recorded at room temperature using Shimadzu UV-VIS 160A spectrophotometer. The analyses were made on samples electroeluted from the pieces of the gels in the presence of 20 mM Tris/maleate, pH 7.0. Chlorophyll *a/b* ratios were determined using the spectrum reconstitution method (Razi Naqvi *et al.*, 1997)

Results

Fractionation of PSII pigment-protein complexes by IEF

When PSII particles were solubilized in the presence of n-HTG they were resolved into LHC II-containing pellets and PSII complexes, collected separately in two centrifugation steps. Following PSII complex treatment with 1% DM and analysis of the solubilized mixture by the ndIEF protocol of Jackowski and Przymusinski (1995) (slightly modified), seven pigmented fractions were resolved in the pH range 3.5–5.0 (Fig. 1), labeled 1–7 in order of decreasing pI value. Fractions 2–7 focused in the form of well defined, narrow bands but the most alkaline fraction (1), consistently migrated as a smear diffused in the pH 4.80–5.00 range. Bands 5–7 had pI values and chl *a/b* ratios almost identical to those previously described for carnation LHC II trimeric subcomplexes A, B and C (Jackowski and Przymusinski, 1995).

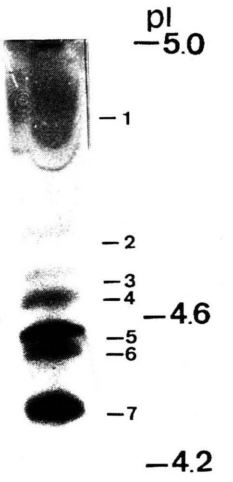

Fig. 1. Fractionation of the PSII complex by ndIEF. PSII complex equivalent of 30 μg chl was resolved in a 0.5 mm-thick, analytical, vertical 7% polyacrylamide slab gel containing 0.47% n-dodecyl-β-D-maltoside. The gel was not stained.

Spectroscopic characterization of the fractions

We recorded the room temperature absorption spectra of each of the fractions. The absorption spectrum of fraction 1 was heavily dominated by chl *a* components at 420, 435, 626.5 and 672.5 nm (Fig. 2). The absence of a distinct peak or even shoulder around 650 nm indicates that the material of this fraction had a low chl *b* content. The distribution of peaks, and the shape of the spectrum was almost identical to that of the PSII reaction center complex purified by others (e.g. Dainese *et al.*, 1990), thus fraction 1 is composed of carnation PSII reaction center complexes.

The absorption spectrum of fraction 2 showed chl *a* absorption peaks at 676.5 and 437.5 nm as well as features thought to be indicative of CP29 *i.e.* the presence of a minor but clearly visible peak attributable to chl *b* in red at 640 nm, and a pronounced Soret peak, which is seen at about

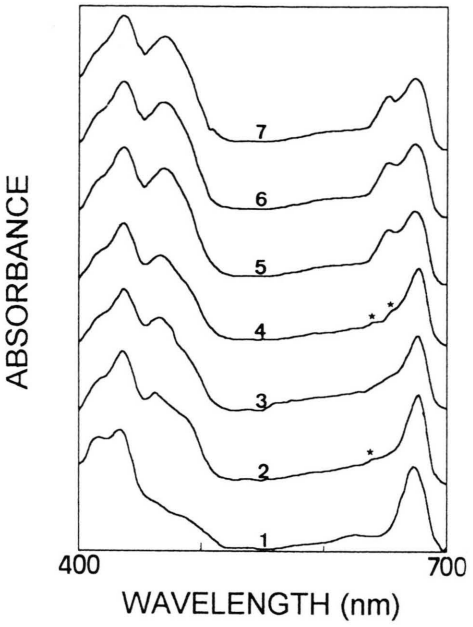

Fig. 2. Absorption spectra at room temperature of fractions 1–7 of the PS II complex. Asterisks indicate the chl b 640 and 653 nm shoulders of CP29 and CP26.

470 nm in other chl *a*/*b*-proteins (Dainese *et al.*, 1990; Henrysson *et al.*, 1989), split into broad 463.5 and 492.5 nm components. Chl *a*/*b* ratio of the fraction 2 was 1.83 : 1. The spectrum of fraction 4 showed a considerable resemblance to the fraction 2 spectrum, including the pattern of chl *a* maxima and the presence of the chl *b* 640 nm peak, but it also displayed a minor peak at 653 nm. Since the presence of chl *b* Qy-region minor peaks both at 640 and around 653 nm is indicative of CP26, we suggest that fraction 4 contained CP26 (chl *a*/*b* ratio was 1.87 for this fraction). The spectrum of fraction 3 was intermediate between those of fractions 2 and 4. Bands 5, 6 and 7 had spectra characteristic of LHC II, with dominating maxima at 673.5, 652, 470 and 437 nm. This provided confirmation that these bands represented LHC II subunits, still present in the PSII complex preparation. Fractions 5, 6 and 7 had similar chl *a*/*b* ratios, 1.02, 0.98 and 1.05, respectively.

Polypeptide composition of the fractions

Next, we analyzed the proteins present in the different fractions using SDS-PAGE and immunoblotting. The polypeptide composition of fractions 1–7 is shown in Fig. 3. The majority of CBB-stainable material of fraction 1 was attributed to psbO, psbB (it migrates as 43 kDa in carnation, data not shown) and psbE as well as to two polypeptides migrating at 30 and 67 kDa, apparently representing psbA/psbD and the psbA/D heterodimer, respectively. Besides these fractions two unidentified polypeptides were observed at 20 and 14 kDa. Fraction 2 (CP29) contained one major (28.8 kDa), and one minor (28.5), polypeptide, and fraction 4 (CP26) contained the unique 26.6 kDa polypeptide. Fraction 3 consisted of both the polypeptides of fraction 2, and the single species of fraction 4. Since fraction 3 appeared to be a mixture of CP29 and CP26, both in terms of polypeptide composition and spectroscopy, we did not analyze this fraction further. In accordance with expectations, fractions 5–7 showed LHC II bands identical to those of LHC II subcomplexes A, B and C, respectively (Jackowski and Przymusinski, 1995). As can be seen in Fig. 4, fractions 2, 4, and

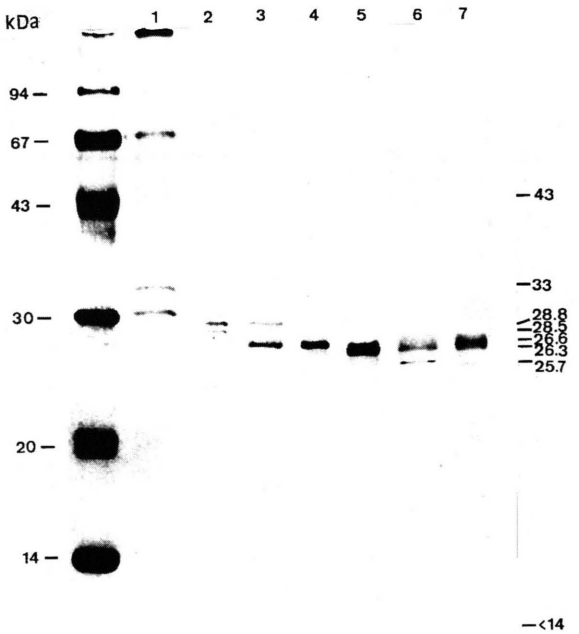

Fig. 3. Polypeptide composition of fractions of the PSII complex. 2 μg chl of polypeptides of fractions 1–7 of the PSII complex were separated by SDS-PAGE and the gel was stained with Coomassie Brilliant Blue G-250. Molecular weight markers are shown on the left. Apparent molecular weights are the averages of more than 20 independent determinations, standard deviations were less than 0.25 kDa.

5–7 seemed to contain CP29, CP26 and the three LHC II subcomplexes highly purified.

To corroborate these assignments, the polypeptides of these fractions were separated electrophoretically, electrotransferred onto nitrocellulose sheets and immunostained with monospecific antibodies raised against Lhcb1, Lhcb2, Lhcb3, (LHC II type 1, 2, and 3, respectively) Lhcb4 (CP29) and Lhcb5 (CP26). As expected, both polypeptides (28.8 and 28.5 kDa) of fraction 2 reacted with the Lhcb4-antibody (Fig. 4), and there was no sign of CP29 in any of the other fractions. The Lhcb5-antibody reacted with fraction 4 (CP26), but there was also a faint reaction with fraction 5 (LHC II subcomplex A). Taken together, this shows that CP29 and CP26 have been purified to homogeneity, that carnation CP26 is a single polypeptide of 26.6 kDa but that CP29 can be separated into two bands, at 28.8 and 28.5 kDa. Thus, the apparent molecular masses of Lhcb4 and Lhcb5 of carnation origin closely resemble the values described for other plant species (Jansson, 1994).

All three LHC II fractions (A, B and C) contained Lhcb1, whereas Lhcb2 was found exclusively in LHC II subcomplex B, and Lhcb3 only in subcomplex C. The Lhcb1 and Lhcb3 apoproteins had an apparent molecular weight of 26.3 kDa, whereas Lhcb2 was present in two forms; of 26.3 and 25.7 kDa. The 26.3 kDa band that reacted with the Lhcb2 antibody could not be Lhcb1 or Lhcb3, since the antibody does not react with LHC II subfractions A and C. On the basis of these results, we identify the 26.3 kDa polypeptide as a mixed population of Lhcb1, 2 and 3 gene products and the 25.7 kD as being encoded by Lhcb2. It can not be directly concluded from these experiments whether subcomplexes A, B and C are in monomeric or trimeric form. To get information on this we subjected the fractions, recovered from ndIEF, to ultracentrifugation in 0.2–0.8M sucrose gradients. As illustrated in Fig. 5, the material of the fractions was recovered as two zones sedimenting with different velocities. The majority of the material was found in the lower zone, presumably representing a trimeric form of LHC II. Even though there is a fraction of the material recovered at the position of monomeric LHC II, these data shows that subcomplexes A, B and C comprise LHC II trimers which , to some extent, have dissociated during the solubilization prior to ultracentrifugation. Thus, subcomplex A appears to be Lhcb1 homotrimer whereas subcomplexes B and C are heterotrimers involving Lhcb1/Lhcb2 and Lhcb1/Lhcb3 pairs, respectively.

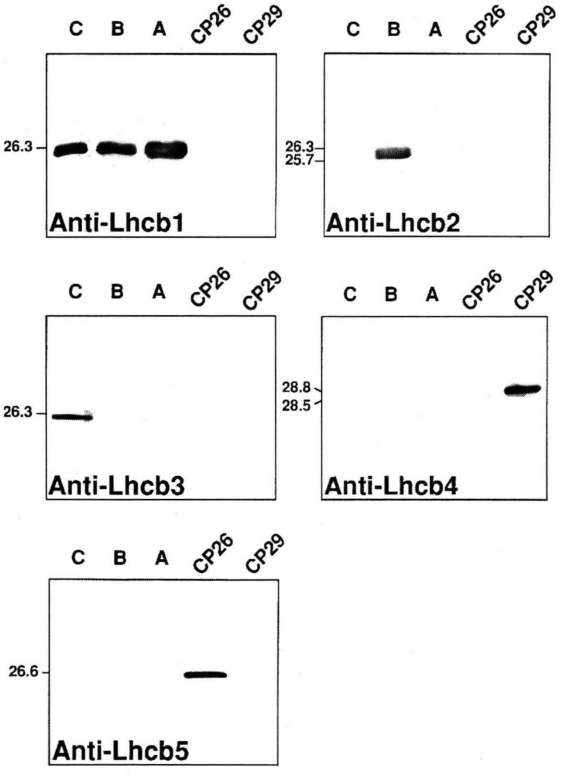

Fig. 4. Immunoblot analysis of fractions of the PSII complex . Polypeptides of CP29, CP26 and LHC II subcomplexes A, B and C were resolved by SDS-PAGE, transferred to nitrocellulose sheet and immunostained with anti Lhcb1, Lhcb2, Lhcb3, Lhcb4 and Lhcb5 antibodies. The amount of the fractions applied per well were normalized to the amount of lutein.

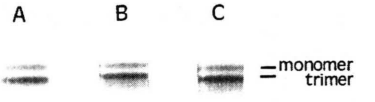

Fig. 5. Resolution of oligomeric forms of LHC II subcomplexes by ultracentrifugation in sucrose gradients. 50 μg chl equivalents of the LHC II subcomplexes A-C electroeluted from ndIEF gels were solubilized in the presence of n-dodecyl-β-D-maltoside, lithium dodecyl sulfate and octyl-β-D-glucopyranoside and spun at 300 000x *g* in 0.2–0.8 M sucrose gradients.

Discussion

Many fundamental characteristics of the LHC proteins are still unknown, largely due to the difficulties in purifying the proteins in their native state, with their pigments still attached. The introduction of novel, non-ionic detergents and utilization of IEF have resulted in better preparations, but a number of basic features such as the precise polypeptide composition of different LHC II trimers remain to be determined. Here we have purified and biochemically characterized CP26, CP29 and three different LHC II trimers from PSII particles prepared from carnation leaves. We believe these preparations to be close to the native state since the samples were received applying a minimal number of preparative steps (ndIEF + electroelution).

We have isolated three different LHC II trimeric complexes, which differ in their apoprotein composition; subcomplex A consists only of Lhcb1, subcomplex B of Lhcb1 and Lhcb2 and subcomplex C of Lhcb1 and Lhcb3. The trimers are purified from PS II complexes that have lost some of their LHC II content but no additional trimers can be detected in carnation thylakoids (Jackowski and Przymusinski, 1995), so we believe that they represent the full set of carnation LHC II trimers. Our data also allow us to draw some conclusions about their polypeptide stoichiometry. The most basic A subcomplex is obviously an Lhcb1 homotrimer, and we believe that subcomplexes B and C consist of two Lhcb1 proteins, together with one molecule of Lhcb2 and Lhcb3, respectively, for the following reasons. Firstly, the immunoreaction with the Lhcb1 antibodies is approximately 50% stronger in subcomplex A than in B and C, on an equal lutein basis. Secondly, densitometric scanning showed that the ratio of 26.3/25.7 kDa polypeptides in subcomplex B is very close to 2:1 (Jackowski and Przymusinski, 1995). Thirdly, we have shown that, in subcomplex C, 1/3 of the CBB-stainable material focused in the 4.20–4.70 range during denaturing IEF and 2/3 at 5.57–5.90 whereas in subcomplexes A and B, all proteins focused between 5.50 and 5.90 (Jackowski and Przymusinski, 1995). As Lhcb3 is considerably more acidic than Lhcb1 and Lhcb2 (Jansson, 1994) the three above findings are all consistent with the polypeptide stoichiometry $(Lhcb1)_3$, $(Lhcb1)_2(Lhcb2)$ and $(Lhcb1)_2(Lhcb3)$ for subcomplexes A, B and C, respectively, but not with other possible stoichiometries.

Different LHC II trimers have been prepared from different species but the differences in apoprotein composition between them have not previously been determined due to the lack of specific antibodies against the polypeptides. In some species, Lhcb1, Lhcb2 and Lhcb3 can be differentiated by their gel mobility but in others this is not possible. In carnation, Lhcb3 and some of the Lhcb2, comigrates with Lhcb1 and in spinach, Lhcb3 comigrates with Lhcb2 (Jansson, unpublished). Since the relative mobility is dependent on electrophoretic conditions and the proteins can exist in different electrophoretic forms, as noted for Lhcb2 above (and discussed in Sigrist and Staehelin, 1994), identification without specific antibodies is, in our opinion, rather unreliable.

The chl *a/b* ratios of subcomplex A, B and C showed only small differences. When other preparations of subcomplex A, B and C was compared (data not shown) no consistent differences were found so our data suggest that the three carnation LHC II trimers have identical pigment composition. We used the novel spectrum reconstruction method (Razi Naqvi *et al.*, 1997) for the determination of pigment content since we consider this to be superior to older methods, but the trends were similar if two wavelength methods like Arnon (1949) or Porra *et al.*, (1989) or HPLC determinations were used. Yet, the chl *a/b* – ratios calculated according to the newest method and the older ones were not the same.

The CP26 (Lhcb5) protein of carnation has an apparent molecular mass of 26.6 kDa and carnation CP29 migrates as two proteins, of 28.8 and 28.5 kDa. We do not know if these are products of different genes or different post-translational modifications like phosphorylation (Croce *et al.*, 1996) or degradation. Such microheterogeneity has previously been reported for CP29 (Allen and Staehelin, 1992; Jansson, 1994). The Chl *a/b* ratio of our CP29 preparation was found to be 1.87. We have thus isolated a CP29 complex with a significantly lower chl *a/b* ratio than previously done but at this stage, we do not know whether this better represents the native CP29 complex. In contrast

to some previous works (e.g. Peter and Thornber, 1991; Bassi *et al.*, 1993; Phillip and Young 1995) our CP29 and CP26 preparations were very similar in chl *a/b* ratio.

The method described in this paper can not be applied to purification of CP24 (Lhcb6), but we have recently been able to partially purify the 22 kD CP24 protein from carnation (Pietrowska and Jackowski, unpublished) using non-denaturing PAGE. In the carnation PSII preparations, CP24 does not seem to be abundant and the majority of it probably dissociates during PSII particle preparation, similar to the PS II preparation reported by Hankamer *et al.* (1997).

We have here described a method for preparing highly purified carnation CP29 and CP26 and three different LHC II trimers in 6–7 h, and have analyzed the apoprotein content of the preparations. By focusing PSII complexes in preparative gels (1.5 mm thick) and using dual- gel apparatus we could obtain 0.2 mg chl equivalents of each CP29 and CP26 in a single IEF run. This enables rapid isolation of large quantities of the pigment-protein complexes, which we believe are suitable for further studies of their bio- and photochemistry.

Acknowledgements

The work was supported by grants from the Polish Committee of Scientific Research, the Swedish Forestry and Agricutural Research Council and Umeå University. The authors gratefully acknowledge the technical assistance of Mrs. Julianna Krysztofiak, the photographic work of Mr. Zygmunt Bartkowiak and the help of Mrs. Lidia Kazmierczak in an initial phase of the study. Finally, Dr. B Bangar Raju is greatly acknowledged for help with calculations according to the spectrum reconstitution method.

Allen J. F., Bennet J., Steinback K. E. and Arntzen C. J. (1981), Chloroplast protein phosphorylation couples plastoquinone redox state to distribution of excitation energy between photosystems. Nature **291**, 25–29.

Allen K. D. and Staehelin L. A. (1992), Biochemical characterization of photosystem II antenna polypeptides in grana and stroma membranes of spinach. Plant Physiol. **100**, 1517–1526.

Armond P. A., Arntzen C. J., Briantais J-M. and Vernotte C. (1976), Differentiation of chloroplast lamellae: light harvesting efficiency and grana development. Arch. Biochem. Biophys. **175,** 54–63.

Arnon D. I. (1949), Copper in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris.* Plant Physiol. **24,** 1–15.

Barbato R., Rigoni F., Giardi M. T. and Giacometti G. M. (1989), The minor antenna complexes of an oxygen evolving photosystem II preparation: purification and stoichiometry. FEBS Lett. **251,** 147–154.

Bassi R., Pineau B., Dainese P. and Marquardt J. (1993), Carotenoid-binding proteins of photosystem II. Eur. J. Biochem. **212,** 297–303.

Bassi R., Rigoni F., Barbato R. and Giacometti G. M. (1988), Light-harvesting chlorophyll *a/b* proteins (LHCII) populations in phosphorylated membranes. Biochim. Biophys. Acta **936,** 29–38.

Chitnis P. R. and Thornber J. P. (1988), The major light-harvesting complex of photosystem II: Aspects of its molecular and cell biology. Photosynth. Res. **16,** 41–63.

Croce R., Breton J. and Bassi R. (1996), Conformational changes induced by phosphorylation in the CP29 subunit of photosystem II. Biochemistry **35,** 11142–11148.

Dainese P. and Bassi R. (1991), Subunit stoichiometry of the chloroplast photosystem II antenna system and aggregation state of the component chlorophyll *a/b* binding proteins. J. Biol. Chem. **266,** 8136–8142.

Dainese P., Hoyer-Hansen G. and Bassi R. (1990), The resolution of chlorophyll *a/b* binding proteins by a preparative method based on flat bed isoelectric focusing. Photochem. Photobiol. **51,** 693–703.

Dreyfuss B. W. and Thornber J. P. (1994), Assembly of the light-harvesting complexes (LHCs) of photosystem II. Monomeric LHC IIb complexes are intermediates in the formation of oligomeric LHC IIb complexes. Plant Physiol. **106,** 829–839.

Enami I., Kamino K., Shen J-R., Satoh K. and Katoh S. (1989), Isolation and characterization of photosystem II complexes which lack light-harvesting chlorophyll *a/b* proteins but retain three extrinsic proteins related to oxygen evolution from spinach. Biochim. Biophys. Acta **977,** 33–39.

Funk C., Schroder W. P., Green B. R., Renger G. and Andersson B. (1994), The intrinsic 22 kDa protein is a chlorophyll-binding subunit of photosystem II. FEBS Lett. **342,** 261–266.

Hankamer B., Nield J., Zheleva D., Boekema E., Jansson S. and Barber J. (1997), Isolation and biochemical characterization of monomeric and dimeric photosystem II complexes from spinach and their relevance to the organisation of photosystem II *in vivo.* Eur. J. Biochem. **243,** 422–429.

Henrysson T., Schröder W. P., Spangfort M. and Akerlund H-E. (1989), Isolation and characterization of the chlorophyll *a/b* protein complex CP29 from spinach. Biochim. Biophys. Acta **977,** 301–308.

Horton P., Ruban A. V. and Walters R. G. (1996), Regulation of light harvesting in green plants. Ann. Rev. Plant Physiol. Plant Mol. Biol. **47**, 655–684.

Jackowski G. (1996), The subcomplex organization of the major chlorophyll *a/b*-protein light-harvesting complex of photosystem II (LHC II) in barley thylakoid membrane. Z. Naturforsch. **51c,** 454–463.

Jackowski G. and Kluck E. (1994), The oligomeric arrangement of the light-harvesting chlorophyll *a/b*-protein complex of photosystem II. Z. Naturforsch. **49c,** 337–342.

Jackowski G. and Przymusinski R. (1995), The resolution and biochemical characterization of subcomplexes of the main light-harvesting chlorophyll *a/b*-protein complex of Photosystem II (LHC II). Photosynth. Res. 43, 41–48.

Jansson S. (1994) The light-harvesting chlorophyll a/b-binding proteins. Biochim. Biophys. Acta **1184,** 1–19.

Jansson S., Stefánsson H., Nyström U., Gustafsson P. and Albertsson P.Å. (1997), Photosystem I and II antenna proteins in the different thylakoid domains. Biochim. Biophys. Acta **1320,** 297–309.

Jansson S., Pichersky E., Bassi R., Green B. R., Ikeuchi M., Melis A., Simpson D. J., Spangfort M., Staehelin L. A. and Thornber J. P. (1992), A nomenclature for the genes encoding the chlorophyll *a/b*-binding proteins of higher plants. Plant Mol. Biol. Rep. **10**, 242–253.

Laemmli UK (1970), Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227,** 680–685.

Larsson U. K., Anderson J. M. and Andersson B. (1987), Variations in the relative content of the peripheral and inner light-harvesting chlorophyll *a/b*-protein complex (LHC II) subpopulations during thylakoid light adaptation and developement. Biochim Biophys. Acta **894,** 69–75.

Lee A. L. C. and Thornber J. P. (1995), Analysis of the pigment stoichiometry of pigment-protein complexes from barley (*Hordeum vulgare*) – The xanthophyll cycle intermediates occur mainly in: the light-harvesting complexes of photosystem I and photosystem II. Plant Physiol. **107,** 565–574.

Peter G. F. and Thornber J. .P (1991), Biochemical composition and organization of higher plant photosystem II light-harvesting pigment proteins. J. Biol. Chem. **266,** 16745–16754.

Phillip D. and Young A. J. (1995), Occurrence of the carotenoid lactucaxanthin in higher plant LHC II. Photosynth. Res. 43, 273–282.

Porra R. J., Thompson W. A. and Kriedemann P. E. (1989), Determination of accurate extinction coefficients and simultaneous equation for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy, Biochim. Biophys. Acta **975**, 384–394.

Razi Naqvi K., Melø T. B. and Bangar Raju B. (1997), Assaying the chromophore composition of photosynthetic systems by spectral reconstitution: Application to the light-harvesting complex (LHC II) and the total pigment content of higher plants. Spectrochemica Acta A **53**, 2229–2234.

Sigrist M. and Staehelin L. A. (1994), Appearance of type 1, 2 and 3 LHCII and LHCI proteins during light-induced greening of barley (*Hordeum vulgare*) etioplasts. Plant Physiol. **104,** 135–145.

Spangfort M., Andersson B. (1989), Subpopulations of the main chlorophyll *a/b* light harvesting complex of Photosystem II – isolation and biochemical characterization. Biochim Biophys Acta **977,** 163–170.