POLYPEPTIDE COMPOSITION OF THE PHOTOSYSTEM I CHLOROPHYLL_{a/b}-PROTEIN FROM THREE DIFFERENT PLANT SPECIES

by

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Photosystem I preparations from *Vicia faba*, *Hordeum vulgare*, and *Zea mays* were analysed for chlorophyll_{a/b}-binding proteins and their polypeptide composition. For this purpose the long wave-length fluorescence emitting band Chl_{a/b}-P3 (LHCI-730) of each species was excised from gels after electrophoresis and the concentrated eluates were subjected to denaturing re-electrophoresis. The polypeptide pattern of Chl_{a/b}-P3 obtained with gradient gels containing 6 M-urea exhibited three bands previously termed B, C, and D (3). Only a polypeptide corresponding in its migration property to D was present in the polypeptide pattern of total thylakoids. A 20 kD polypeptide which corresponds to D is missing in the chlorophyll *b*-less barley mutant *chlorina f2*. It is concluded that D binds pigments and may represent the apoprotein of Chl_{a/b}-P3. The authenticity and function, respectively, of the polypeptides B and C need further elucidation.

I. INTRODUCTION

The existence of a second light-harvesting chlorophyll_{a/b}-protein complex (LHCI) associated with photosystem I (PSI) has been recently established (3, 4, 5, 6, 7, 11, 12, 13). Mild electrophoretic conditions yield a high molecular weight complex containing the reaction centre for PSI, the core antenna and the chlorophyll *b* containing light harvesting antenna. This complex termed chlorophyll_a-protein 1* (Chl_a-

PI*) exhibits a 732 to 735 nm fluorescence emission from the bound chlorophyll (1). After gentle dissociation three chlorophyll binding bands can be isolated by electrophoresis or sucrose density gradient centrifugation. These are chlorophyll_a-protein 1, the chlorophyll_{a/b}-protein 3 or LHC-730 and chlorophyll_{a/b}-protein 4 or LHC-680 (2, 3). The latter two bands emit fluorescence at 730 and 680 nm, respectively. Chl_{a/b}-P3 (LHC-730) is in maize thyla-

Abbreviations: Chl = chlorophyll; Chl-P = chlorophyll-protein; DTT = dithiothreitol; kD = kilodalton; LHCI = light harvesting complex of photosystem I; LHCII = light harvesting complex of photosystem II; PSI = photosystem I; SDS = sodium dodecyl sulphate; Tricine = N-(tris-(hydroxymethyl)-methyl)glycine; Tris = tris-(hydroxymethyl) amino methane.

koids composed of 3 polypeptides with apparent molecular masses of 24 (B), 22.5 (C), and 21 (D) kD. $\text{Chl}_{a/b}$ -P4 (LHC-680) yields two polypeptides with a mass of 25 (A) and 20.5 (E) kD, while the Chl_a -P1 consists of one or two polypeptides with a mass of 67 kD. The high molecular weight Chl_a -P1* contains additionally 12 to 15 polypeptides with molecular masses between 19 and 5 kD (2, 3).

In the present paper two additional species, *Vicia faba* and *Hordeum vulgare*, are analysed for chlorophyll $_{a/b}$ -binding proteins and the composition of the light harvesting components of Chl_a -P1*.

2. MATERIALS AND METHODS

2.1. Chloroplast isolation

Leaves of *Vicia faba*, *Hordeum vulgare*, and *Zea mays* from plants cultivated in a growth chamber at 20 °C in continuous light were homogenized in 0.1 M-Tricine/NaOH, pH 7.8, containing 0.4 M-sorbitol. Chloroplast isolation was performed by 5 min centrifugation at $500 \times g$ and 10 min at $2000 \times g$. After hypotonic shock with 10 mM-Na-pyrophosphate, pH 7.4, in an ice bath the pellets were washed two times using the same buffer. The pellets were resuspended at a chlorophyll concentration of $2 \text{ mg} \cdot \text{ml}^{-1}$ in 65 mM-Tris/HCl, pH 6.8, 10% glycerol and 5 mM-dithiothreitol. One ml samples were frozen with liquid nitrogen and stored at minus 80 °C until further analysis.

2.2. Thylakoid membrane dissociation

500 μl of 50 mM-Tricine/NaOH buffer, pH 8.0, containing octylglucoside plus SDS (20:1) was pre-cooled in an ice bath. An equal volume of thawed thylakoid suspension was added to give an octylglucoside:SDS:chlorophyll ratio of 20:1:1. After 5 min incubation the solution was centrifuged at $150,000 \times g$ for 10 min and the supernatant was loaded on the top of 2 mm slab gels.

2.3. Electrophoresis

Two different electrophoretic systems were applied in the course of these experiments. To keep the degree of chlorophyll-protein denaturation low, the first and second electrophoretic steps were performed with the buffer system of Laemmli at 4 °C using homogeneous gels of 10% (running gel) and 4% (stacking gel), respectively. Prior to re-electrophoresis of Chl_a -P1*, the excised band was incubated for 10 min with 100 mM-NaCl in an ice bath and then placed on the top of a new gel with 100 mM-NaCl in the stacking gel. The chlorophyll-protein bands obtained after re-electrophoresis were excised and either frozen or homogenized, eluted, and concentrated using Sartorius Collodium Bags SM 13200.

To analyse the polypeptide composition of excised chlorophyll-protein bands and eluates, electrophoresis was performed under denaturing conditions at 20 °C using a Tris-glycine/Tris-

Figure 1. Chlorophyll-protein bands obtained by low-temperature electrophoresis of thylakoid membranes dissociated with octylglucoside plus SDS (β -octyl glucoside:SDS:Chl = 20:1:1).

Figure 2. Re-electrophoresis of $\text{Chl}_a\text{-P1}^*$ following mild dissociation with NaCl yields, beside free pigments, three chlorophyll-protein bands with *Vicia faba* and two bands each with *Hordeum vulgare* and *Zea mays* (a). The green bands are termed $\text{Chl}_a\text{-P1}$, $\text{Chl}_{a/b}\text{-P3}^*$, and $\text{Chl}_{a/b}\text{-P3}$. The bands marked by arrows exhibit 730 nm fluorescence emission. Coomassie-Blue staining shows between one (*Zea mays*) and three (*Hordeum vulgare*) additional polypeptide bands marked by arrow heads (b).

sulfate buffer system (System IV in MACHOLD et al. 1979). For this purpose 10% to 20% gradient gels of 1 mm thickness with and without 6 M-urea were applied.

3. RESULTS

3.1. Chlorophyll-proteins of thylakoids and PSI-preparations

Preparative electrophoresis (2.3) of dissociated thylakoid membranes from *Vicia faba*, *Hordeum vulgare*, and *Zea mays* yielded eight chlorophyll-protein bands each, as shown in Figure 1. The band patterns obtained are in good correspondence with those found previously (3, 8, 9, 10).

In the present experiments attention was focussed on the chlorophyll-protein(s) associated with the band termed $\text{Chl}_a\text{-P1}^*$ (Figure 1) or CP1a (3). Dissociation of $\text{Chl}_a\text{-P1}^*$ and re-electrophoresis at low temperature yielded the reaction center chlorophyll-protein of PSI, $\text{Chl}_a\text{-P1}$, and either one or two additional chlorophyll $_{a/b}$ -protein bands termed $\text{Chl}_{a/b}\text{-P3}$ and $\text{Chl}_{a/b}\text{-P3}^*$ (Figure 2). These exhibited long-wavelength fluorescence emission.

One chlorophyll $_{a/b}$ -protein band ($\text{Chl}_{a/b}\text{-P3}$) was obtained in the present experiments with $\text{Chl}_a\text{-P1}^*$ from *Hordeum vulgare* and *Zea mays*

and two ($\text{Chl}_{a/b}\text{-P3}$ and $\text{Chl}_{a/b}\text{-P3}^*$) from *Vicia faba*. The two bands from *Vicia faba* gave the same fluorescence emission and polypeptide composition as shown below and in agreement with previous results (3). The fact that two bands were found with maize in previous experiments and only one here may be the result of small variations in the procedures applied.

In some experiments with maize an additional green band was found close to the free pigment

Figure 3. In some experiments with *Zea mays* a chlorophyll $_{a/b}$ -protein band exhibiting 680 nm fluorescence emission was obtained. This chlorophyll-protein was interpreted as a connecting antenna (BASSI et al. 1985)

Figure 4a. Polypeptide patterns of thylakoid membranes from *Vicia faba*, *Hordeum vulgare*, and *Zea mays*.

Figure 4b. Polypeptide composition of $\text{Chl}_a\text{-P1}^*$ from the three plant species. The patterns were obtained by re-electrophoresis of eluted and concentrated $\text{Chl}_a\text{-P1}^*$.

Figure 5. Polypeptide composition of eluates from $\text{Chl}_{a/b}$ -P3 compared with that of complete membranes. The patterns were obtained with gradient-gels in the presence (a) or absence (b) 6 M-urea. The arrows point to the major polypeptide D which is postulated to represent the apoprotein of LHCI chlorophyll $_{a/b}$ -protein. The arrow-heads refer to the apoproteins of LHCII.

zone exhibiting fluorescence emission at 680 (Figure 3). This band corresponds to the chlorophyll $_{a/b}$ -protein 4 (LHCI-680) of a connecting antenna complex (3).

Coomassie Blue-staining of the gels in Figure 2 yields, in addition to the green bands, pigment-free polypeptides marked by arrow heads (Figure 2b). Only one polypeptide band was found with *Zea mays* but two with *Vicia faba* and three with *Hordeum vulgare* revealing the existence of species specific differences.

3.2. Species specific differences of the polypeptide patterns from thylakoid membranes and PSI-preparations

Previous experiments with LHCII from *Vicia faba* and *Hordeum vulgare* exhibited clear species specific differences with respect to the electrophoretic behaviour of the apoproteins (8). Since analogous differences were expected for the apoprotein(s) of LHCI, comparable experiments were performed with complete thylakoid membranes and PSI preparations from *Vicia*

faba, *Hordeum vulgare*, and *Zea mays*.

In Figure 4a are presented the polypeptide patterns of isolated thylakoids after dissociation with SDS and electrophoresis on gradient gels with and without 6 M-urea. Next to the track of the pattern from *Vicia faba* the presently known apoproteins are marked. With both procedures applied, a good resolution of the 20 kD region was obtained, but there are distinct differences among the three species in the electrophoretic mobility of individual polypeptides.

In comparison with the polypeptides of the membranes, re-electrophoresis of excised, eluted, and condensed $\text{Chl}_{a/b}$ -P1* shows the apoprotein of $\text{Chl}_{a/b}$ -P1 and up to 15 diffuse bands (Figure 4b). From the stainable material accumulated especially in the upper part of the gels it is apparent that the procedure, even though it was performed at low temperature, causes protein denaturation, and perhaps an unspecific aggregation of some of the membrane polypeptides.

3.3. Polypeptides associated with the chlorophyll_{a/b}-protein(s) of LHCI

The chlorophyll_{a/b}-protein bands of *Vicia faba*, *Hordeum vulgare*, and *Zea mays* designated as $\text{Chl}_{a/b}$ -P3 and $\text{Chl}_{a/b}$ -P3* in Figure 2 were excised, eluted and concentrated as described under Materials and Methods. The samples were warmed for 5 min at 60 °C in the presence of 5% DTT and re-electrophoresed together with control samples of total thylakoid membranes treated in the same way.

The results of these experiments are demonstrated in Figure 5. The polypeptide patterns of $\text{Chl}_{a/b}$ -P3 from *Vicia faba* and *Zea mays* (slots 2 and 10) obtained with gradient gels containing 6 M-urea exhibited three bands previously termed B, C, and D (3). Re-electrophoresis of the same samples with gradient gels not containing urea yielded only two bands, a major one and a minor one (slots 4 and 12). In contrast to *Vicia faba* and *Zea mays* the polypeptide patterns obtained from *Hordeum vulgare* with the two procedures show the same pattern viz. a major band with a diffuse zone above (slots 6 and 8). Within the diffuse zone of slot 8 two weak bands are visible.

Comparable experiments were performed

Figure 6. Stoichiometric ratio of the two major apoproteins, $\text{Chl}_{a/b}$ -AP2a and $\text{Chl}_{a/b}$ -AP2b, of LHCI from *Vicia faba*. The preparations termed $\text{Chl}_{a/b}$ -P2 represent eluates of the corresponding bands shown in Figure 1. All samples compared exhibit the same stoichiometry with respect to these two apoproteins.

Figure 7. Polypeptide pattern of $\text{Chl}_{a/b}$ -P3 from *Vicia faba* as compared with that of Chl_a -P1* obtained under identical conditions. In contrast to the major chlorophyll $_{a/b}$ -apoproteins of LHCII (Figure 6), the patterns and intensities of the polypeptides B and C differ among the samples compared.

Figure 8. Polypeptide composition of the two $\text{Chl}_{a/b}$ -P3 forms with different electrophoretic mobility from *Vicia faba*. Although the polypeptide pattern of $\text{Chl}_{a/b}$ -P3 does not correspond to bands obtained from dissociation of complete membranes, it is identical with the pattern of $\text{Chl}_{a/b}$ -P3*.

Figure 9. Re-electrophoresis of the excised $\text{Chl}_{a/b}$ -P3 bands from *Vicia faba*, *Hordeum vulgare*, and *Zea mays* with two different gel systems. The polypeptides were stained by silver.

with monomeric and oligomeric chlorophyll $_{a/b}$ -protein of LHCII using the two bands termed $\text{Chl}_{a/b}$ -P2 and $\text{Chl}_{a/b}$ -P2** of *Vicia faba* in Figure 1. They were excised and prepared in the same way as described for $\text{Chl}_{a/b}$ -P3. Re-electrophoresis of both samples yielded the two apoproteins $\text{Chl}_{a/b}$ -AP2a and 2b in the same relative proportion. The pattern of the 20 kD and 30 kD polypeptides corresponded exactly to that of the control (Figure 6).

Of the three polypeptide bands revealed upon re-electrophoresis of $\text{Chl}_{a/b}$ -P3 in *Vicia faba*, only one polypeptide corresponding in its migration property to D is present in the pattern of total thylakoids (Figure 5). Eluates of $\text{Chl}_{a/b}$ -P3 and the complex $\text{Chl}_{a/b}$ -P1* were investigated using identical conditions (Figure 7). All samples were heated to 60 °C for 5 min, frozen with liquid nitrogen and heated once more. It was found that in both cases the polypeptide D was

Figure 10. Polypeptide patterns of thylakoid membranes from *Hordeum vulgare* wild type and *chlorina f2*. Arrows indicate those polypeptides reduced in the mutant.

released and migrated in a position comparable to a band in the pattern of total thylakoids. The polypeptides B and C are likewise released from both bands but again polypeptide bands of comparable stoichiometry are not found in the thylakoid patterns. Whether the polypeptides B and C are authentic or artefacts cannot be decided from the present experiment.

In Figure 8 the composition of the two bands emitting 730 nm fluorescence but migrating at

different positions, $\text{Chl}_{alb}\text{-P3}$ and $\text{Chl}_{alb}\text{-P3}^*$ are compared. In both cases polypeptides D, C and B are released, indicating that $\text{Chl}_{alb}\text{-P3}^*$ is a dimer or oligomer of $\text{Chl}_{alb}\text{-P3}$.

The procedures of protein elution and sample concentration as applied in the experiments discussed have the advantage that high protein concentrations can be obtained. Since the results achieved did not allow a conclusive interpretation, an additional experiment with excised chlorophyll-protein bands was performed. The excised gel strips were layered onto the top of a gradient gel and covered with 10% acrylamide. Due to the low protein content, silver staining was necessary to make polypeptides visible. Figure 9 reveals the results obtained with excised $\text{Chl}_{alb}\text{-P3}$ from *Vicia faba*, *Hordeum vulgare*, and *Zea mays*. If gradient gels containing 6 M-urea were used for re-electrophoresis one polypeptide band was found in the position of 20 kD. The pattern of *Hordeum vulgare* shows two additional bands in the upper gel region. Using gels without urea, one polypeptide band was obtained with *Vicia faba* and two bands with *Hordeum vulgare* and *Zea mays*. The latter had an additional weak band in the position where the undissociated $\text{Chl}_{alb}\text{-P3}$ is expected to be.

For further characterization of the polypeptides B, C, and D found in $\text{Chl}_{alb}\text{-P3}$ preparations the barley mutant *chlorina f2* was included into the experiments. This mutant, which completely lacks chlorophyll *b*, is known to be deficient in some of the corresponding chlorophyll *b* carrying apoproteins. This mainly concerns the two major apoproteins, $\text{Chl}_{alb}\text{-AP2a}$ and $\text{Chl}_{alb}\text{-AP2b}$, of LHCII. Figure 10 indicates three additional polypeptides of 20, 20.5, and 28 kD (marked by arrows) which are also affected. The 20.5 and 28 kD polypeptides are associated with LHCII. This can be concluded from experiments where dissociation of LHCII is reduced by Triton-X 100 incubation of thylakoid membranes at low temperature (Figure 11). The 20 kD polypeptide appears identical with D. The absence of this polypeptide in the pattern of the mutant indicates its involvement in binding chlorophyll *b*. From this it can be concluded that D represents the true apoprotein of $\text{Chl}_{alb}\text{-P3}$. The authenticity and function, respectively, of polypeptides B and C need further elucidation.

Figure 11. Polypeptide composition of LHCII from *Hordeum vulgare* isolated at low temperature.

4. DISCUSSION

In the experiments using re-electrophoresis of chlorophyll containing bands it was found with all three plant species analyzed that only polypeptide D (Figures 5, 7, and 8) was consistently associated with chlorophyll_{a/b}-protein of LHCI. One interpretation is that this polypeptide alone binds the pigments and represents the true apoprotein of Chl_{a/b}-P3. This is in conformity with the results obtained in experiments with chlorophyll *b*-less barley mutant *chlorina f2*. The polypeptide pattern of this mutant completely lacks D indicating its close relationship to chlorophyll *b*. The authenticity and function, respectively, of the polypeptides B and C also found with Chl_{a/b}-P3 preparations is not yet clear. The large difference between the electrophoretic mobilities of D and its holoprotein Chl_{a/b}-P3 might indicate that the latter is a dimer composed of two molecules D plus pigments. Accordingly, Chl_{a/b}-P3* can be conceived of as a tetramer.

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