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POLYPEPTIDE COMPOSITION AND SPECTRAL PROPERTIES OF LIGHT-HARVESTING CHLOROPHYLL *a/b*-PROTEIN COMPLEXES FROM INTACT AND TRYPSIN-TREATED CHLOROPLAST THYLAKOID MEMBRANES

KARL-HEINZ SÜSS and EBERHARD BRECHT

Zentralinstitut für Genetik und Kulturpflanzenforschung, Akademie der Wissenschaften der D.D.R., 4325 Gatersleben (G.D.R.)

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Summary

The polypeptide composition and spectral properties of isolated light-harvesting chlorophyll *a/b*-protein complexes from intact and trypsin-treated thylakoid membranes of *Hordeum vulgare* and *Vicia faba* are compared. The LHCP complexes consist of four distinct polypeptides with molecular weights between 21 000 and 25 000 occurring in equal relative amounts in the whole polypeptide spectra of thylakoid membranes. It is shown indirectly that the two major polypeptides very probably belong to different chlorophyll-proteins. The loss of a small segment from both polypeptides during trypsin digestion of thylakoids does not substantially alter the spectral properties and cation-mediated aggregation of isolated LHCP complexes.

Up to now three protein complexes enriched in the light-harvesting chlorophyll *a/b*-protein (LHCP), the *P* 700 chlorophyll *a*-protein of PS I and a chlorophyll *a*-protein of PS II have been isolated from anionic detergent extracts of higher plant thylakoid membranes [1]. The oligomeric LHCP complex has no photochemical activity and is thought to be involved in the cation-mediated processes of grana stacking, distribution of freeze-fracture particles in the plane of thylakoid membranes and regulated distribution of

Abbreviations: SDS, sodium dodecyl sulfate; LHCP, light-harvesting chlorophyll *a/b*-protein; PS I and PS II, Photosystem I and II.

excitation energy to both photosystems [2]. The polypeptide composition and molecular weights of LHCP polypeptides has been a matter of some confusion; one [3], two [4] or more [5] components were found to be associated with the isolated complex. Moreover, it is not clear yet which of these components are apoproteins of chlorophyll-proteins. Previous studies showed a selective alteration of polypeptides associated with the LHCP complex during trypsin digestion of thylakoids accompanied with changes of membranes properties [4, 6]. In this report we describe the spectral properties and polypeptide composition of isolated LHCP complexes from intact and trypsin-treated thylakoid membranes.

Thylakoid membranes of *Vicia faba* and *Hordeum vulgare* were isolated [7] and purified in the presence of 10 μM phenylmethylsulfonylfluoride. Trypsin digestion of *V. faba* thylakoids for 3 h was carried out as outlined previously [4]. The nondigested membrane material was extracted five times using 1 M NaCl or 8 M urea in 50 mM Tris-HCl, pH 8.0, containing 0.5 mg/ml soya-bean trypsin inhibitor. Extraction with 8 M urea and washing of intact thylakoids was performed as described [4]. The LHCP complexes were isolated by a modified method as described [8]. Intact and trypsin-treated thylakoids were disintegrated with 3% Triton X-100 in 5 mM Tris-HCl, pH 8.3, with or without soya-bean trypsin inhibitor (0.1 mg/ml). The ratio of detergent to chlorophyll was 10 ml/g. Extract with about 15 mg chlorophyll was layered on a short DEAE-cellulose column (3 × 6 cm), preequilibrated with 0.05% Triton X-100 in 5 mM Tris-HCl, pH 8.3, and washed with two times the column volume of the same buffer. The LHCP was eluted by washing the columns with 0.3 M NaCl in the above mentioned buffer. $MgCl_2$ was added to the eluates yielding a final concentration of 20 mM. After incubation at 4°C for 3 h the aggregated LHCP complexes could be sedimented at 5000 × *g* for 20 min. Small traces of *P*-700 chlorophyll *a*-protein were separated by redissolving the pellets in 5 mM Tris-HCl, pH 8.3, containing 0.05% Triton X-100 and repeating the procedure of Mg^{2+}-mediated aggregation of LHCP complexes two or three times. The purification procedure can be completed in 8 h with a maximal yield of 20% on a total chlorophyll basis. In order to analyse the polypeptide composition and to determine the molecular weights, thylakoid membranes or isolated LHCP complexes were disintegrated in a solution of 50 mM Na_2CO_3, 30 mM dithiothreitol, 10% sucrose and 2% lithium dodecyl sulfate (pH 9.0) and fractionated by lithium dodecyl sulfate-polyacrylamide gel electrophoresis. The gel slabs were made of a 6% acrylamide stacking gel of a linear concentration gradient of acrylamide (8—18%) accompanied by a 0.5—10% sucrose gradient. The ratio of acrylamide/*N'*,*N*-methylene bisacrylamide was 19:1 (gel system 1). The high-resolution gel system 2 was composed as gel system 1, but contained 5 M urea additionally. Electrophoresis was performed at a constant current of 20 mA for about 20 h at 4°C.

The LHCP complexes from *V. faba* and *H. vulgare* isolated and purified here exhibited very similar room temperature absorption spectra with chlorophyll *a*-peaks at 438 and 676 nm (*Vicia*) or 677 nm (*Hordeum*) as well as chlorophyll *b*-peak at 472.5 and 652 nm (Fig. 1). These peaks at long wave-

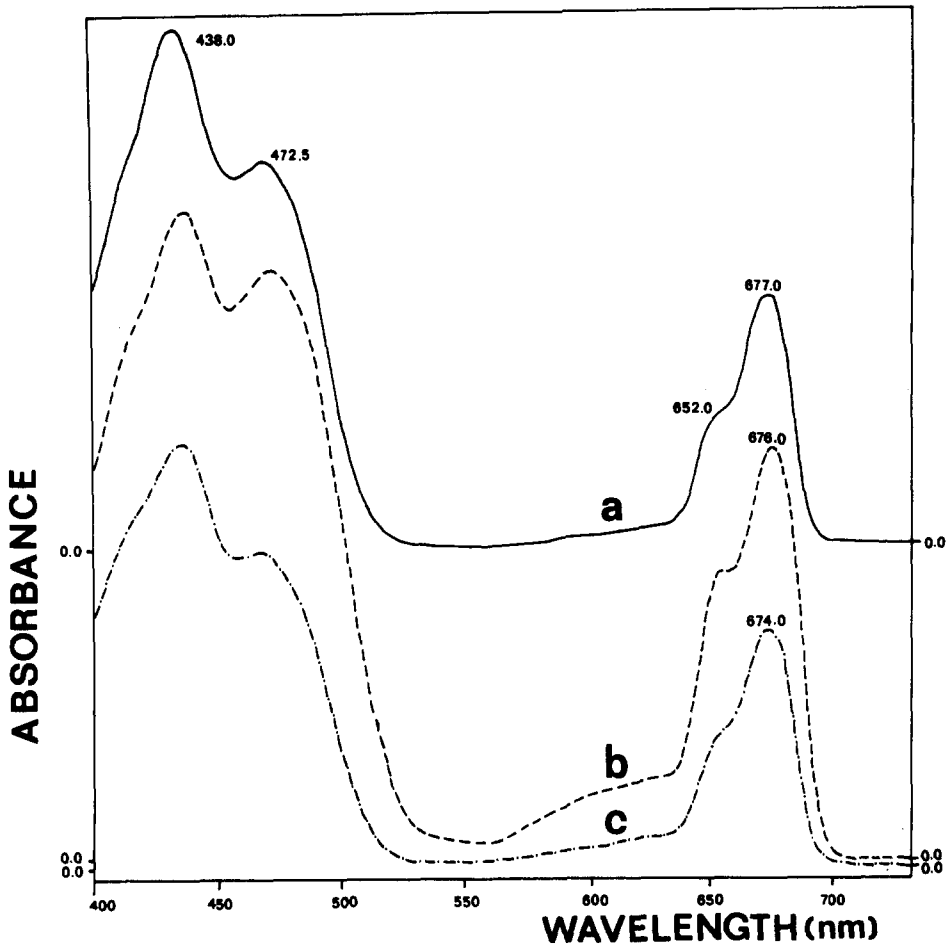

Fig. 1. Absorption spectra of isolated LHCP complexes from intact thylakoid membranes of *Hordeum vulgare* (a), *Vicia faba* (b) and trypsin-treated thylakoid membranes of *Vicia faba* (c), suspended in 5 mM Tris-HCl buffer, pH 8.3.

lengths indicate the intactness of the spatial arrangement of the pigment molecules. Besides, carotenoids could be detected (peak at 486 nm) by means of second derivative spectra (not shown), recorded with a special spectrophotometer for light-scattering samples [9]. The minimum chlorophyll *a/b* ratios of the LHCP complexes from *Vicia* and *Hordeum* were determined to be 1.21 and 1.29 respectively, by the method of Arnon [10]. Similar chlorophyll *a/b* ratios have been reported for the LHCP complexes from *Pisum* [11] and *Hordeum* [12, 13]. Fig. 2 shows the results of a high-resolution lithium dodecyl sulfate/urea polyacrylamide gel electrophoresis of isolated LHCP complexes and intact thylakoid membranes. The LHCP complexes from *Vicia* and *Hordeum* could be fractionated into four polypeptide bands, termed a to d. In contrast to the well separated components of the LHCP complex from *Vicia* the polypeptides from *Hordeum* had more similar electrophoretic mobilities. In the presence of 0.1% lithium dodecyl sulfate and 5 M urea the molecular weights of LHCP polypeptides were determined to be

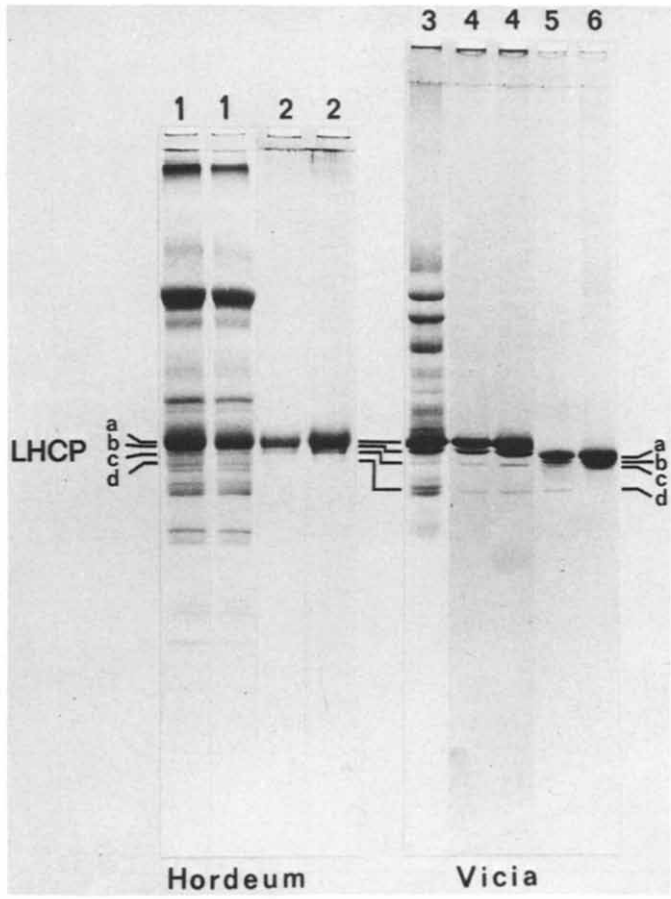

Fig. 2. High resolution lithium dodecyl sulfate/urea-polyacrylamide gel electrophoresis of intact thylakoid membranes (1, *Hordeum vulgare*; 3, *Vicia faba*) and isolated LHCP complexes from intact (2, *Hordeum vulgare*; 4, *Vicia faba*) and trypsin-treated *Vicia faba* thylakoids washed with 1 M NaCl (5) or 8 M urea (6) before isolation of the LHCP complexes.

25 000, 24 000, 22 000 and 21 000 (*Vicia*) and 25 000, 24 500, 24 000 and 22 500 (*Hordeum*). The polypeptides a and b occur in a ratio of about 2:1 (*Vicia*) and 1:1 (*Hordeum*) as estimated by densitometry of the gels. Since equal relative amounts of the components a to c *(Vicia)* and a to d *(Hordeum)* were obtained from the complete polypeptide spectra of the corresponding thylakoid membranes the conclusion is drawn that these polypeptides are structurally associated with the light-harvesting assembly of higher plant thylakoids. The present findings also confirm that a successfull separation of the different polypeptides can only be achieved by lithium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis in the presence of urea; otherwise the major LHCP components migrate as a single band. The piment binding polypeptides of the LHCP complexes were analyzed by two-dimensional lithium dodecyl sulfate polyacrylamide gel electrophoresis (Fig. 3) using the gel system 1 in the first and gel system 2 in the second dimension. Under the condition of gel system 1 the LHCP complexes were separated into a holoprotein band (H) with an approximated molecular weight of 27 000 and three *(Vicia)*

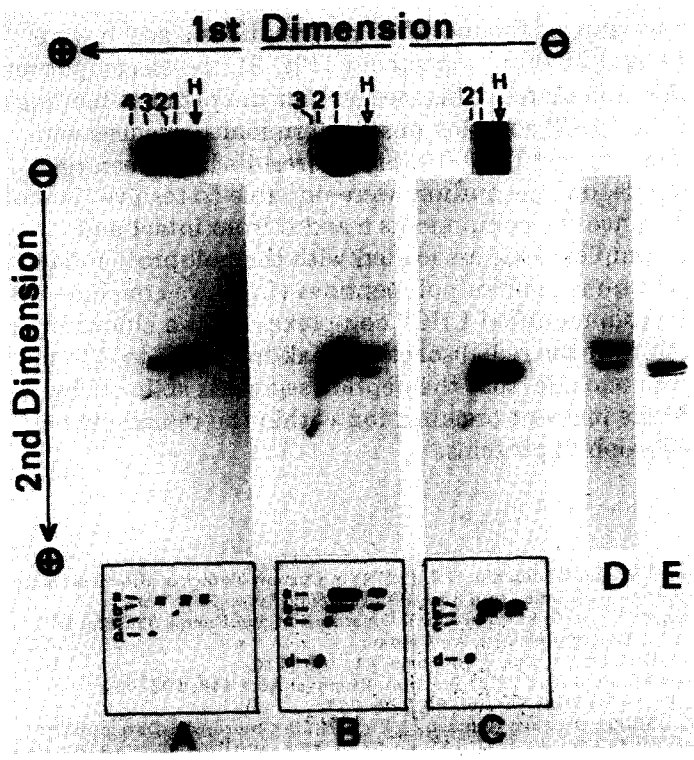

Fig. 3. Two dimensional lithium dodecyl sulfate-polyacrylamide gel electrophoresis of LHCP complexes from intact thylakoid membranes of *Hordeum vulgare* (A), *Vicia faba* (B) and from trypsin-treated thylakoids of *Vicia faba* (C). The gels D and E show a re-electrophoresis of the holoprotein band H of B and C, respectively, on gel system 2.

or four *(Hordeum)* chlorophyll-free proteins zones in the range of 24 000 to 20 000. The holoprotein bands contain chlorophyll a and b as well as carotenoids as determined by spectrophotometry of the gel segments (not shown). When analyzed in the second dimension of electrophoresis (gel system 2), the holoprotein zone and chlorophyll-free protein bands 1 *(Vicia)* and 1 as well as 3 *(Hordeum)* (Fig. 3, A, B) were found to consist of the polypeptides a and b, occurring in a quantitative ratio as mentioned above. Therefore, the pigment-free zones can be considered as apoprotein bands. Since the polypeptides a and b form common holo- as well as apoprotein bands under the conditions of gel system 1, the shape and extrinsic change of those detergent-polypeptide complexes can be assumed to be similar and seem to be responsible for the same electrophoretic behaviour. So we propose that the polypeptides a and b of the LHCP complexes are apoproteins of two different chlorophyll-proteins. Since the same gel systems had been used their apoproteins can be assumed to be identical with the polypeptides previously termed AP II a and AP II b [4].

Two different LHCP complexes with respect to the polypeptide composition could be isolated from trypsin-treated *Vicia* membranes. One of them isolated from NaCl-washed thylakoids contained four different polypeptides (a to d), whereas three (a to c) were found after extraction with 8 M urea

(Fig. 2). The molecular weights of the modified polypeptides a and b were determined to be 23 500 and 23 000, respectively (Fig. 3); the electrophoretic mobilities of c and d were nonaltered. This means, that the pigment binding components a and b of the LHCP complex possess terminal sequences with molecular weights of 1500 (a) and 1000 (b). Similar results have been reported for *Pisum* thylakoids, but the authors were not able to resolve the both major polypeptides [6]. Since the components a and b from intact and trypsin-modified LHCP complexes are associated with the holoprotein band H (Fig. 3 B, C) and show the same ratio of polypeptides (2:1), we conclude the identity of them. The trypsin-modified LHCP complexes show a chlorophyll *a/b* ratio of 1.22 and a shift of the red absorption peak from 677 to 674 nm (Fig. 1). Accordingly, we conclude, that the peptide segments split off by trypsin affect only little the pigment organization within the remaining part of the corresponding chlorophyll-proteins.

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