IDENTIFICATION OF CHLOROPLAST THYLAKOID MEMBRANE POLYPEPTIDES

ATPase complex (CF_1–CF_0) and light-harvesting chlorophyll *a/b*–protein (LHCP) complex

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1. Introduction

Thylakoid membranes of higher plants and green algae can be resolved by SDS–polyacrylamide gradient gel electrophoresis into nearly 30 major polypeptides and additional minor components with mol. wt ⩽70 000 [1,2]. A detailed knowledge of the biological properties of these polypeptides is essential in order to get a better insight into such important problems as the protein composition, the organization of proteins within the thylakoids, and the biogenesis of thylakoid membranes. Since the native conformations and biological properties of the components are destroyed in the presence of ionic detergents it has been attempted to identify their function by the following ways:

(i) Analysis of the polypeptide composition of functionally well characterized photosynthetic mutant membranes, the missing component(s) being correlated with the deleted function [1,3–7];
(ii) Correlating the appearance of polypeptides during the greening process of plastids with the development of photosynthetic activities [4,8];
(iii) Checking the influence of antibodies against isolated membrane polypeptides on partial photosynthetic reactions [9–11];
(iv) Co-electrophoresis of the whole membrane polypeptide mixture with authentic membrane proteins as reference substances [6,12–15].

The following components have been identified with certainty: the subunits $\alpha, \beta, \gamma, \delta$ and ϵ of coupling factor CF_1 [12,14,15], the cytochromes *f*, *b*-559, *b*-563 [12,15], the apoproteins of 3 chlorophyll *a*–proteins [5,16,17] as well as of 3 chlorophyll *a/b*–proteins [2,15,18]. Up to now there is no consensus concerning the number and identity of polypeptides of the light-harvesting chlorophyll *a/b*–protein complex as well as the membrane sector CF_0 of the ATPase complex (CF_1–CF_0).

This paper describes the identification of polypeptides of the entire ATPase complex and the LHCP complex in the gel electrophoretic spectrum of *Vicia faba* thylakoid membranes. The ATPase complex was isolated from Triton X-100 extracts of thylakoids by immunoprecipitation with antibodies against CF_1. The whole protein complex contains 9 different polypeptides 5 of them coinciding with the CF_1 subunits $\alpha, \beta, \gamma, \delta$ and ϵ.

The δ subunit was found to be present in much higher amount in the ATPase complex compared with the isolated CF_1. The molecular weights of the CF_0 components, the smallest being the proteolipid, were determined to be 19 000, 17 000, 16 500 and 7500, respectively. The isolated LHCP complex contains 4 different polypeptides termed a–d with mol. wt 21 000–25 000. The polypeptides a and b are very probably apoproteins of 2 different chlorophyll–proteins. All polypeptides of the ATPase complex and the LHCP complex correspond to major peaks in the membrane polypeptide profiles. Moreover, the positions of the subunits of the cytochromes *f*, *b*-559, *b*-563 are also described.

Abbreviations: CF_1, chloroplast coupling factor 1; CF_0, membrane sector of the chloroplast ATPase complex; LHCP complex, light-harvesting chlorophyll *a/b*–protein complex; LiDS, lithium dodecyl sulfate

2. Materials and methods

2.1. *Preparation of thylakoid membranes, coupling factor CF_1 and of antibodies against CF_1*

Envelope- and stroma-free chloroplast thylakoid membranes from *Vicia faba* were prepared as in [19]. The isolation and purification of coupling factor CF_1 consisting of the subunits $\alpha, \beta, \gamma, \delta$ and ϵ as well as immunization of rabbits with pure CF_1 was carried out as in [12]. The immunogammaglobulin (IgG) fractions were precipitated from the antisera with ammonium sulfate at 50% of saturation, dissolved in 5 mM Tris–HCl (pH 8.0) and dialyzed against the same medium before freeze-drying. The antibodies showed only crossreactions with the α and β subunit of CF_1 as determined by two-dimensional crossed immunoelectrophoresis [20].

2.2. *Immunoprecipitation of the ATPase complex and isolation of the proteolipid*

Thylakoid membranes (containing 5 mg chlorophyll) were dissolved in 1 ml 5 mM Tris–HCl buffer (pH 8.0) containing 5% Triton X-100 at 4°C. The solution was centrifuged at 20 000 × *g* for 15 min and the clear supernatant was used for immunoprecipitation. Supernatant (1 ml) was slowly added from a microsyringe to 1 ml agitated solution containing 100 mg IgG dissolved in the buffer mentioned above. After incubation for 3 h at 25°C, the immunoprecipitate formed was sedimented by centrifugation at 6000 × *g* for 10 min. The pellet obtained was suspended in 4 ml 2% Triton X-100 in 5 mM Tris–HCl (pH 8.0) and layered over 2 ml solution containing 5% sucrose, 0.5% Triton X-100 and 5 mM Tris–HCl (pH 8.0) in a small glass centrifuge tube. The immunoprecipitate was sedimented by centrifugation at 6000 × *g* for 10 min some green material being concentrated at the interface of both solutions. The supernatant was discarded and the pellet was washed twice with 5 mM Tris–HCl (pH 8.0) containing 0.5% Triton X-100 before the extraction of the proteolipid or the elucidation of the polypeptide composition.

The proteolipid (Pl) was extracted at 0°C by suspending the immunoprecipitates of the ATPase complex (5 mg/ml) in a 1:1 (v/v) mixture of chloroform/methanol for 20 min. After centrifugation at 10 000 × *g* for 10 min, the pellet was discarded and the pure proteolipid could be obtained from the supernatant after evaporation of the organic solvent.

2.3. *Isolation of the LHCP complex*

The LHCP complex was isolated from Triton X-100 extracts of thylakoid membranes and purified including DEAE-cellulose chromatography and MG^{2+} precipitation as in [18].

2.4. *Analytical procedures*

Thylakoid membrane polypeptides and isolated membrane proteins were analyzed by LiDS/urea electrophoresis in slab gels (180 × 160 × 1 mm) containing an 8–18% acrylamide concentration gradient as in [18]. The membranes were disintegrated and the isolated proteins dissociated into their subunits with 2% LiDS, 8 M urea and 10 mM dithiothreitol dissolved in 50 mM Na_2CO_3. For solubilization of the immunoprecipitates dithiothreitol was omitted to prevent dissociation of the light and heavy antibody chains. Chlorophyll was determined as in [21] and protein concentration estimated as in [22].

3. Results and discussion

3.1. *The immunoprecipitated ATPase complex*

Thylakoid membranes are quantitatively soluble at Triton X-100 to chlorophyll ratios (v/w) of >10. The ATPase complexes could be immunoprecipitated from the detergent extracts of thylakoid membranes from different higher plants (*Vicia faba, Spinacia oleracea, Hordeum vulgare*) and the alga *Chlorella pyrenoidosa* using antiserum against coupling factor CF_1 from *Vicia faba* which contained antibodies crossreacting with the α and β subunit of this protein. These antibodies inhibited the ATPase activity of the purified CF_1 (unpublished). The immunoprecipitates were frequently contaminated with traces of the P700 chlorophyll *a*–protein of PSI as well as the LHCP complex, which probably resulted from an incomplete solubilization of membrane fragments enriched in ATPase complex. These impurities are removable by the method in section 2.

The polypeptide profile of the immunoprecipitated ATPase complex from detergent-solubilized thylakoid membranes of *Vicia faba* as well as the purified CF_1 is shown in fig.1c,d. Nine different polypeptides of the ATPase complex were clearly separated by LiDS/urea–polyacrylamide gel electrophoresis, 5 of them coinciding with the CF_1 subunits $\alpha, \beta, \gamma, \delta$ and ϵ with mol. wt 59 000, 55 000,

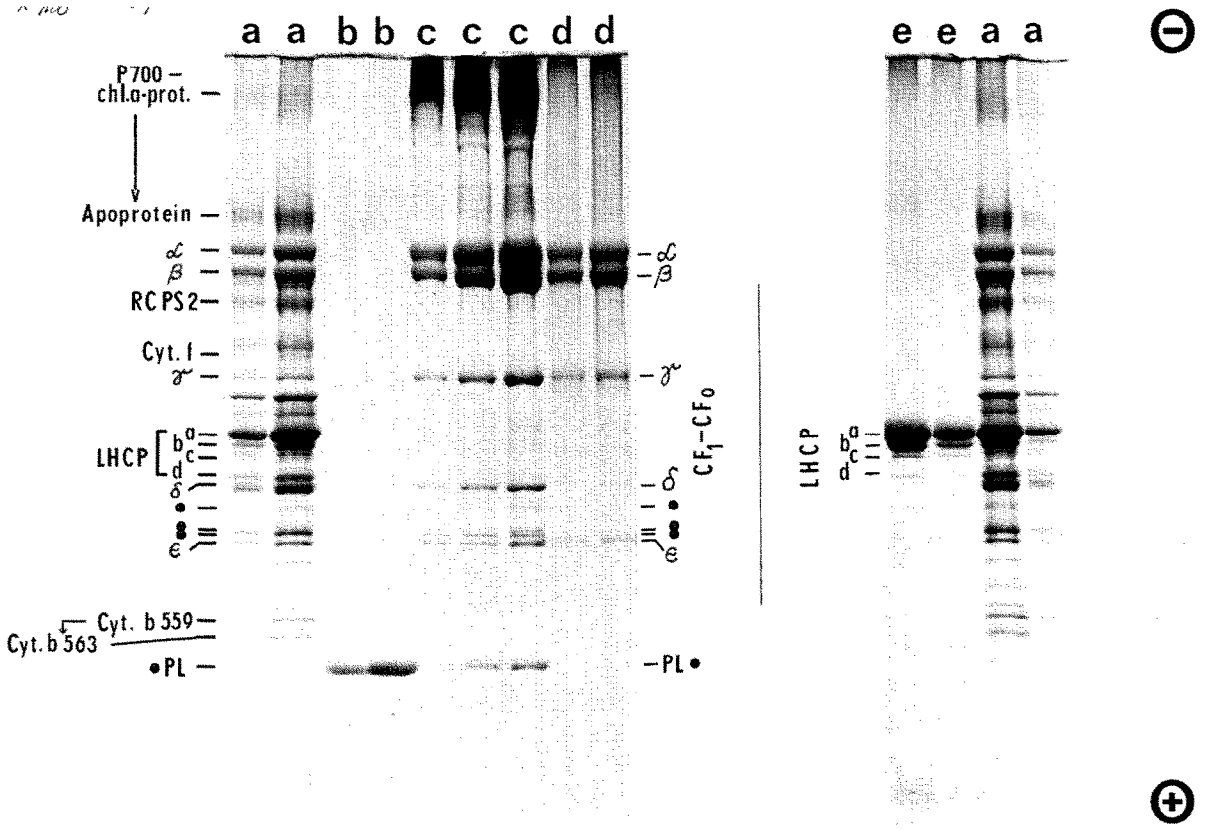

Fig.1. Co-electrophoresis of thylakoid membrane polypeptides and isolated proteins on LiDS/urea polyacrylamide gradient gels: (a) Thylakoid membrane polypeptides; (b) proteolipid (PL) extracted from immunoprecipitates of the ATPase complex; (c) immunoprecipitated ATPase complex; (d) purified coupling factor CF_1; (e) isolated light-harvesting chlorophyll *a*/*b*–protein complex. The bands identified in the thylakoid membrane polypeptide profile are indicated on the left (cyt. = cytochrome). The polypeptides marked by black dots are equivalent to the components associated with the membrane sector CF_0 of the ATPase complex.

37 000, 21 000 and 16 000, respectively [12]. The heavily stained protein bands moving above the α subunit of CF_1 can be attributed to the undissociated rabbit immunogammaglobulins. If compared with the purified CF_1, the ATPase complex contains the same relative amounts of subunit molecules except the δ subunit, the latter being present in 3-times higher amount (fig.2b,c). This strongly suggests that the binding of the δ subunit to CF_1 is weakened during extraction and/or purification of the protein. The same result has been obtained for beef heart F_1 ATPase [23]. The 4 additional polypeptides of the immunoprecipitated ATPase complex with mol. wt 19 000, 17 000, 16 500 and 7500 are associated with the membrane sector CF_0. Since the low molecular weight polypeptide of CF_0 is quantitatively extractable from immunoprecipitates with a 1:1 (v/v) chloroform/methanol mixture it very probably represents the proteolipid of the ATPase complex (fig.1b). A small polypeptide with the same electrophoretic mobility was extracted from intact thylakoid membranes by the procedure in [24] which yields the chloroplast membrane dicyclohexylcarbodiimide-binding protein.

A similar polypeptide composition has been reported for the ATPase complex purified from cholate/octylglycoside extracts of spinach chloroplasts [25]. This ATPase complex also consists of 4 different CF_0 polypeptides, 3 of them termed CI–III. These components move between the δ and ε subunit of CF_1 during SDS gel electrophoresis. The relatively low amounts of the polypeptides CI and CII

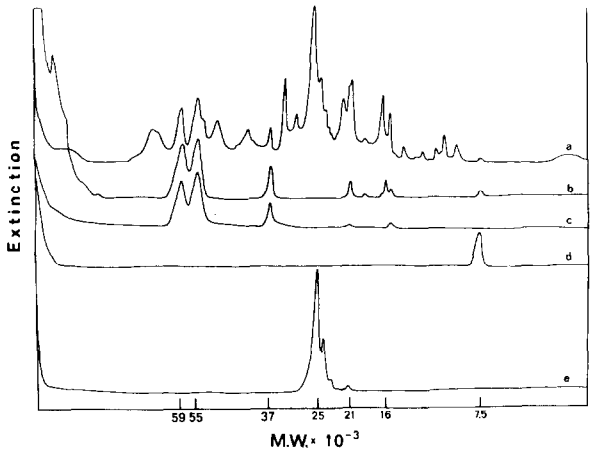

Fig.2. Densitograms of the polypeptides of: (a) thylakoid membranes; (b) the ATPase complex plus immunogammaglobulin bands; (c) the purified coupling factor CF_1; (d) the proteolipid (PL) extracted from the immunoprecipitated ATPase complex; (e) the light-harvesting chlorophyll *a*/*b*–protein complex obtained on LiDS/urea–polyacrylamide gradient gels.

present in this preparation, compared with the corresponding polypeptides (mol. wt 17 000 and 16 500) of our immunoprecipitates, is probably due to the incomplete solubilization of these CF_0 components from thylakoid membranes by the detergents used.

Although similarities between number, molecular weights and relative amounts of subunits among the immunoprecipitated ATPase complexes from intact thylakoid membranes of *Vicia faba* and *Spinacia oleracea* have been shown, differences were observed for *Hordeum vulgare*. Besides the proteolipid, the ATPase complex of the latter contains ⩾4 instead of 3 CF_0 components with electrophoretic mobilities between those of the δ and ϵ subunit of CF_1. Moreover, a polypeptide with mol. wt 10 000 is found in addition to the full set of subunits in the ATPase complex from *Hordeum etioplasts*. This component disappears ~5 h after the beginning of the light-induced plastid development (K.-H. S., in preparation). These results and those in [25] indicate, therefore, that polypeptides with mol. wt 42 000, 32 000 and 23 000 do not belong structurally to the chloroplast ATPase complex as concluded in [26–28].

3.2. *The LHCP complex*

The LHCP complex isolated from Triton X-100 extracts of thylakoid membranes as in [18] consists of 4 different polypeptides termed a–d (fig.1e). The stoichiometric ratios of the components a–c were found to be the same in the isolated LHCP complexes and in the thylakoid membranes (fig.1,2). The molecular weights of the polypeptides were estimated by LiDS/urea–polyacrylamide gel electrophoresis to be 25 000 (a), 24 000 (b), 22 500 (c) and 21 000 (d) [18]. LiDS gel electrophoresis of the isolated LHCP complexes in the absence of urea yields up to 3 chlorophyll *a* and *b* as well as carotenoid-containing holoprotein bands with mol. wt 27 000–70 000. They altogether contain the polypeptides a, b in a stoichiometric ratio of ~2:1 [15]. This result was taken as indirect evidence that both polypeptides are apoproteins of different chlorophyll–proteins with very similar electrophoretic mobilities of their monomeric and oligomeric forms. The question remains open, whether components c,d also represent apoproteins of additional chlorophyll–proteins.

3.3. *Identification of the bands in the thylakoid polypeptide spectra*

As shown in fig.1, 19 different polypeptide bands have been identified in the gel electrophoretic spectra of *Vicia faba* chloroplast thylakoid membranes using purified and characterized membrane proteins or protein complexes as references. Nine of them belong to the ATPase complex (CF_1–CF_0) and 4 to the LHCP complex. The smallest polypeptide of the membrane seems to be the proteolipid of the ATPase complex with mol. wt ~7500, the largest being the apoprotein of the P700 chlorophyll *a*–protein of PSI. The isolation, characterization and identification of further polypeptides associated with the cytochromes *f*, *b*-559 and *b*-563, the chlorophyll *a*–protein termed RCPS 2 (which is probably a component of the PSII reaction center) and the P700 chlorophyll *a*–protein of PSI have been described [12,15].

It should be noted that 2 different polypeptides were found to be associated with the pure cytochrome *b*-563. The component with the slightly higher molecular weight comigrated with the apoprotein of cytochrome *b*-559 during LiDS/urea–polyacrylamide gel electrophoresis. However, the question remains open, whether some other bands identified functionally in the membrane polypeptide profile also represent single polypeptides or a mixture of different polypeptides with the same electrophoretic mobilities.

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