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THE LIGHT-HARVESTING CHLOROPHYLL *a*/*b* · PROTEIN COMPLEX OF THE GREEN ALGA *ACETABULARIA MEDITERRANEA*

ISOLATION AND CHARACTERIZATION OF TWO SUBUNITS

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SUMMARY

In the green alga *Acetabularia mediterranea* a light-harvesting chlorophyll *a*/*b* · protein complex of 67 000 daltons has been found which contains two polypeptide chains of 21 500 and 23 000 daltons. These two polypeptides were isolated on a preparative scale and were further characterized by several different methods. Both polypeptides proved to be very similar. While their amino acid and sugar compositions as well as their immunochemical properties were almost identical the tryptic peptides and the cyanogen bromide fragments of the two polypeptides revealed minor but significant differences. The 67 000-dalton chlorophyll *a*/*b* · protein complex and its two polypeptide components were compared to the light-harvesting chlorophyll *a*/*b* · protein of higher plants.

INTRODUCTION

Many attempts have been made to explore the molecular organization of the chloroplast membrane [1, 2]. One approach is to isolate the different components of the chloroplast membrane and to characterize them in more detail before attempting to describe the interaction of the different constituents within the membrane [3, 4].

Chloroplast membranes can be solubilized in the presence of detergents and their components may be separated electrophoretically. Under the experimental conditions originally described by Ogawa et al. [5] and Thornber et al. [6] two chlorophyll-binding proteins were, among other chloroplast membrane proteins, separated by electrophoresis in polyacrylamide gels. They were termed complex I and complex II [6]. They differ from each other in their chlorophyll *a*/*b* ratios, their relationship to Photosystems I and II and their apparent molecular weights. Complex II contains chlorophyll *b* as well as chlorophyll *a*, it is probably part of Photosystem II, and its apparent molecular weight has been determined to be between 27 000 and 35 000 [7]. Recently evidence has been presented suggesting that complex II is not required for photosynthetic electron transport [8] and Thornber has renamed this complex the light-harvesting chlorophyll *a*/*b* · protein [7].

In previous work [9] we have shown that in the chloroplast membrane of the green alga *Acetabularia mediterranea* a chlorophyll-protein is present which seemed to be identical with the light-harvesting chlorophyll *a/b* · protein in that it contained both chlorophyll *a* and *b*, it was part of Photosystem II and its apparent molecular weight of 21 500 is similar to that reported for the light-harvesting chlorophyll *a/b* · protein of other plants. However, in addition to the 21 500-dalton chlorophyll-binding protein a second major chlorophyll · protein complex of 67000 daltons was detected in *Acetabularia*, which has the same chlorophyll *a/b* ratio as the 21 500-dalton chlorophyll · protein and which is also found exclusively in a Photosystem II fraction. Furthermore, we could show that the 67 000-dalton chlorophyll · protein complex can be dissociated and that the 21 500-dalton chlorophyll · protein is one of two electrophoretically distinct components [9]. The second component of 23 000 daltons seems to be free of chlorophyll under the experimental conditions used [9]. We have assumed that the 67 000-dalton chlorophyll · protein complex of *Acetabularia* is a higher molecular weight light-harvesting chlorophyll *a/b* · protein complex and thus we have referred to the 21 500-dalton chlorophyll · protein as the chlorophyll-binding subunit of the 67 000-dalton light-harvesting chlorophyll *a/b* · protein complex [9].

In a previous paper the localization of the two subunits of the 67 000-dalton chlorophyll *a/b* · protein complex has been described [10]. The 21 500-dalton chlorophyll-binding subunit seems to be buried within the lipid layer of the membrane while the 23 000-dalton subunit is at least partially exposed to the membrane's surface. Thus, both subunits not only have different apparent molecular weights but differ also with regard to their localization within the membrane. In the present study we describe in more details the chemical structure of the two isolated subunits of the 67 000-dalton chlorophyll *a/b* · protein complex.

METHODS

Isolation of chloroplast membrane proteins

For the isolation of the 21 500- and 23 000-dalton subunits of the 67 000-dalton chlorophyll *a/b* · protein complex of *A. mediterranea*, the fraction B_{EDTA} [10] was solubilized in the presence of 0.2 % sodium dodecyl sulfate and separated electrophoretically as described previously [9]. The 67 000-dalton chlorophyll *a/b* · protein complex was eluted from the gel [9] and dissociated into the two subunits by extracting the protein three times with 90 % acetone. The protein material was dried under vacuum, redissolved with 2 % dodecyl sulfate, 65 mM Tris · HCl (pH 6.8) and dansylated according to the method of Talbot and Yphantis [11]. The dansylated protein was separated from the free dansyl chloride on a Sephadex G-25 column which was equilibrated with 0.1 % dodecyl sulfate, 65 mM Tris · HCl (pH 6.8). The two subunits were separated on a linear 10–15 % polyacrylamide gradient gel ($40 \times 20 \times 0.4$ cm) using the electrophoresis system of Neville [12]. The protein bands were monitored directly in the gel during electrophoresis as described by Talbot and Yphantis [11] and were isolated as described earlier [9].

The concentrated protein solutions were dialyzed against water to remove the excess of free dodecyl sulfate. The tightly protein-bound dodecyl sulfate was removed by the method of Weber and Kuter [13]: The protein for production of antisera was dialyzed against a suspension of Dowex 1X 2 (3 g/100 ml) in 1 % Triton X-100, 6 M

urea at room temperature for 12–16 h. Under these conditions the protein remained soluble. Otherwise, the protein was dialyzed against a suspension of Dowex 1 X 2 in distilled water. The resulting protein precipitate was frozen and lyophilized. This protein material was used for the amino acid and sugar determinations, the trypsin treatment and the cyanogen bromide cleavage. In all cases the purity of the isolated 21 500- and 23 000-dalton polypeptides was tested by re-running aliquots of the separated polypeptides on an analytical sodium dodecyl sulfate polyacrylamide gel.

Cyanogen bromide treatment

The lyophilized protein was dissolved in 70 % formic acid (2 mg protein/ml of final vol.) and cyanogen bromide was added up to a concentration of 20 mg cyanogen bromide per mg protein. The incubation was performed at 2 °C for 20 h. The incubation was stopped by diluting the sample 10-fold with distilled water. The frozen sample was lyophilized, resuspended in water and freeze-dried a second time. This protein material was then electrophoretically separated on a linear 13–19 % polyacrylamide gradient gel in the presence of 0.1 % dodecyl sulfate.

Trypsin treatment

The lyophilized protein was suspended in 0.1 M ammonium bicarbonate (1 mg/ml final vol.) and trypsin (1 mg/ml 0.001 M HCl) was added up to a final concentration of 20 μg/ml. The sample was incubated at 37 °C for 22 h. The incubation was stopped by the addition of acetic acid and the frozen sample was lyophilized.

Peptide mapping was performed by combined electrophoresis and chromatography in two dimensions on silica gel thin layer plates (Macherey-Nagel, silicagel Sil G-25) according to the method of Wieland and Georgopoulos [14].

Amino acid composition analysis

Amino acid analysis was done according to the method of Takemoto and Bogorad [15], except that the iodine-acetamide treatment was omitted.

Sugar determination

The isolated polypeptides of 21 500 and 23 000 daltons were hydrolyzed according to the method of Lehnhardt and Winzler [16] and the sugars determined as described by Sawardeker et al. [17]. The sugar estimation was done by Dr. I. Fromme, Max-Planck-Institut für Immunbiologie, Freiburg.

Preparation of antisera and immunochemical methods

Full-grown rabbits were injected subcutaneously in the back with 1 mg of the isolated acetone-extracted 67 000-dalton chlorophyll *a*/*b* · protein complex of *A. mediterranea* in 6 M urea, 1 % Triton X-100. The protein solutions (1 ml) were mixed before injection with an equal volume of Freund's adjuvant (Difco Lab., Detroit, Michigan) containing 1 mg of methylated bovine serum albumin. Booster injections of 0.5 mg protein in incomplete Freund's adjuvant were administered in the back four weeks after the initial injections and the rabbits were bled one week later.

The antisera were purified by ammonium sulfate precipitation, dialyzed against rabbit saline and were stored frozen until ready for further use.

Ouchterlony double-diffusion analyses were performed in small petri dishes

filled with 1 % agarose, 0.1 % Triton X-100 in rabbit saline. Incubation was performed at 20 °C for 3–5 days in the dark. The antisera were tested against the two isolated dansylated polypeptides of 21 500 and 23 000 daltons, a mixture of both polypeptides which had not been dansylated, and the isolated 125 000-dalton chlorophyll · protein of *A. mediterranea* [18], all acetone-extracted and dissolved in 1 % Triton X-100, 6 M urea.

Protein determination

Protein was determined by the method of Lowry et al. [19].

RESULTS AND DISCUSSION

Isolation of the 67 000-dalton chlorophyll a/b · protein complex of A. mediterranea and its two subunits

Until recently chloroplast membranes of higher plants and green algae, including *Acetabularia*, were thought to contain only 10–20 different polypeptides [7]. However, application of sodium dodecyl sulfate polyacrylamide gel electrophoresis with high resolution has now revealed that the dissolved chloroplast membranes contain a higher number of polypeptides [9, 20]. Because of the large number of chloroplast membrane proteins, isolation and purification of an individual protein like the chlorophyll · protein complex is extremely difficult. This problem can be partially alleviated by prefractionating the chloroplast membrane before the isolation of a chloroplast membrane protein from one of the resulting submembrane fractions. Such an approach has been used successfully by Hoober and Stegeman [21] and Machold and coworkers [22]. We have prefractionated the chloroplast membrane of *A. mediterranea* by the combined treatment with EDTA and Triton X-100 into the two submembrane fragments B_{EDTA} and C_{EDTA} which are related to Photosystem II and Photosystem I, respectively [10]. After solubilizing fraction B_{EDTA} in a solution of 0.2 % sodium dodecyl sulfate, only four protein bands were separated electrophoretically: a broad protein band of approximately 47 000 daltons and the undissociated chlorophyll *a/b* · protein complex of 67 000 daltons and its two subunits, the chlorophyll-binding polypeptide of 21 500 daltons and the 23 000-dalton polypeptide (Fig. 1C) [10].

The 67 000-dalton chlorophyll *a/b* · protein complex could easily be isolated. After electrophoresis, gel sections containing this chlorophyll · protein complex were cut out. The 67 000-dalton chlorophyll *a/b* · protein complex was eluted and re-electrophoresed. Under these conditions the 67 000-dalton chlorophyll *a/b* · protein complex was dissociated into the two subunits of 23 000 and 21 500 daltons (Fig. 1A, B).

One may argue that the two subunits of the 67 000-dalton chlorophyll *a/b* · protein complex are only two different electrophoretic forms of the same polypeptide and that the difference in electrophoretic mobility of these two proteins is caused by their binding or non-binding to chlorophyll. We could exclude this possibility in two different ways. Firstly, the 23 000- and 21 500-dalton polypeptides were still present when the isolated 67 000-dalton chlorophyll *a/b* · protein complex was dissolved with 1 % dodecyl sulfate at 90 °C (Fig. 1A) or extracted with acetone prior to reelectrophoresis. Under these conditions all the chlorophyll was dissociated from the protein.

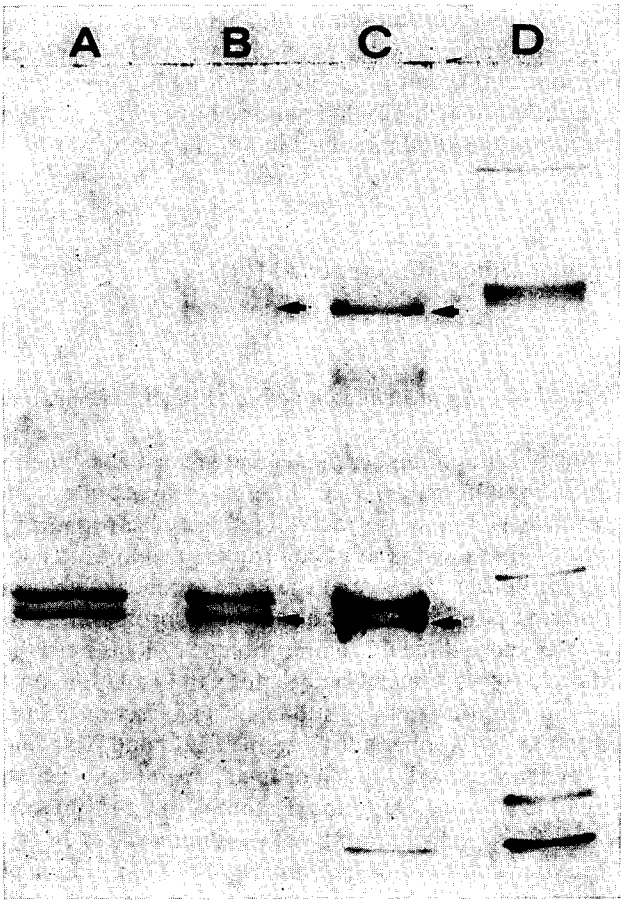

Fig. 1. Re-electrophoresis of the 67 000-dalton chlorophyll *a*/*b* · protein complex of *A. mediterranea* isolated in the presence of 1 % sodium dodecyl sulfate at room temperature and incubated for 1 min at 90 °C (A) or in the presence of 0.2 % sodium dodecyl sulfate at 2 °C (B). (C) Electrophoresis of fraction B_{EDTA} [10]. (D) Marker proteins β-galactosidase (130 000), bovine serum albumin (69 000), carbonic anhydrase (29 000), ribonuclease (13 700) and cytochrome *c* (12 500). The arrows indicate the position of the chlorophyll-binding proteins. The gel was stained with Coomassie Blue.

Secondly, it was possible to separate the 21 500- and 23 000-dalton subunits by preparative gel electrophoresis. After reelectrophoresis of the isolated 21 500-dalton subunit only this isolated polypeptide but not the 23 000-dalton component was recovered on the gel, and vice versa (Fig. 2A–C). Thus, the 23 000-dalton polypeptide does not seem to be derived from the 21 500-dalton polypeptide during solubilization and appears to represent a distinct polypeptide.

The separation of the two polypeptides was based on differences in the apparent molecular weights. Since the two subunits of 21 500 and 23 000 daltons were derived from the 67 000-dalton chlorophyll *a*/*b* · protein complex which was isolated by virtue of its own molecular weight from the submembrane fraction B_{EDTA}, it seems highly unlikely that other polypeptides could contaminate the two isolated subunits.

From the densitometric tracing curves of the Coomassie Blue-stained proteins

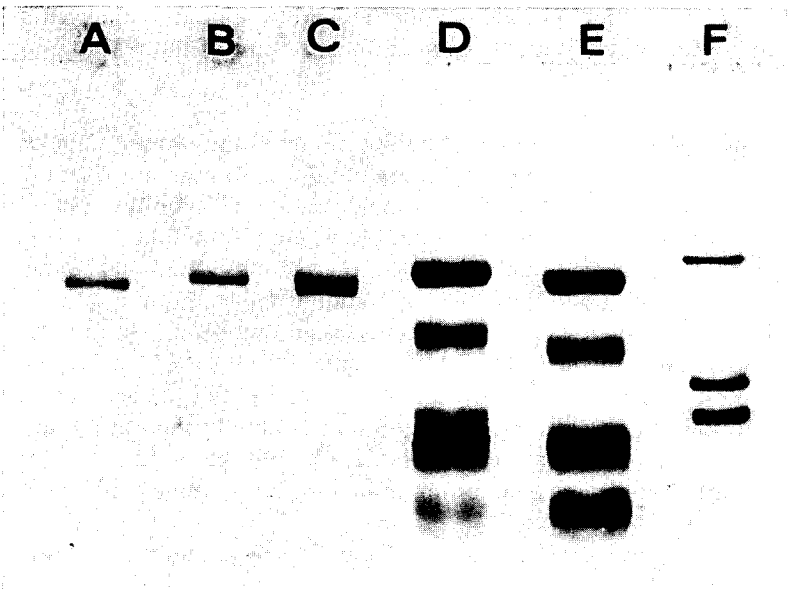

Fig. 2. Electrophoresis of the cyanogen bromide fragments of the 23 000-(D) and the 21 500-(E) dalton subunits of the 67 000-dalton chlorophyll *a*/*b* · protein complex of *A. mediterranea*. (A–C) Separation of the untreated subunits: (A) The isolated polypeptide of 21 500 daltons, (B) the isolated polypeptide of 23 000 daltons and (C) coelectrophoresis of the two isolated polypeptides. (F) Marker proteins carbonic anhydrase (29 000), ribonuclease (13 700) and cytochrome *c* (12 500). The gel was stained with Coomassie Blue.

the molecular ratio of the 23 000- and the 21 500-dalton subunits in the 67 000-dalton chlorophyll *a*/*b* · protein complex had been estimated previously to be 2 : 1 [9]. This ratio would correspond to a minimum molecular weight of 67 500 for the undissociated chlorophyll · protein complex which would be in agreement with the value of 67 000 calculated from the electrophoretic mobility of the chlorophyll · protein complex. In the present study the two subunits of the 67 000-dalton chlorophyll *a*/*b* · protein complex were isolated by preparative gel electrophoresis and the polypeptides eluted from the gel were measured directly by the method of Lowry et al. [19]. By this method we could not confirm our previous result. Instead, we found that the molecular ratio of the 23 000- to the 21 500-dalton polypeptide was 1 : 1 (1.03 : 1$\pm$0.025). The apparent molecular weight of the chlorophyll *a*/*b* · protein complex as determined by its relative electrophoretic mobility on sodium dodecylsulfate gels seems to be higher than expected from the molecular weights of its two separated subunits. A similar anomalous behavior has been reported for the chlorophyll · protein complex I of *Chlamydomonas* [23].

Characterization of the two subunits of the 67 000-dalton chlorophyll a/b · protein complex of A. mediterranea

Both subunits of the 67 000-dalton chlorophyll *a*/*b* · protein complex of *A. mediterranea* were isolated on a preparative scale as described under Methods and their chemical structure was characterized by the determination of the amino acid and

sugar composition, by their immunochemical properties and by separating the peptide fragments obtained by trypsin and cyanogen bromide treatment of the two isolated subunits.

(*a*) *Amino acid analysis.* The amino acid compositions of the two subunits and the undissociated chlorophyll *a*/*b* · protein complex of 67 000 daltons are shown in Table I and were compared with that of the 125 000-dalton chlorophyll · protein complex of Photosystem I which had been described elsewhere [18]. While the content of hydrophobic amino acids is remarkably similar in the 125 000- and 67 000-dalton chlorophyll · proteins the amounts in particular of histidine, lysine, arginine, proline and glycine found in each of the two proteins are significantly different. In contrast, the amino acid compositions of the two subunits of the 67 000-dalton chlorophyll *a*/*b* · protein complex were so similar that any difference which may exist between the two polypeptides can only be revealed by other methods (Table I). The amino acid composition of the 67 000-dalton chlorophyll *a*/*b* · protein complex and its two subunits is very similar to that of the light-harvesting chlorophyll *a*/*b* · protein of spinach (Table I) and a variety of other plants [7].

(*b*) *Determination of carbohydrates.* Some of the chloroplast membrane components have been found to be glycoproteins [24–26], containing up to 12 % carbohydrates [25]. Following the electrophoretic separation of the two isolated subunits of

TABLE I

Amino acid composition (mol %) of the 67 000-dalton chlorophyll *a*/*b* · protein complex and its two subunits and of the 125 000-dalton chlorophyll · protein complex of *A. mediterranea* and of the light-harvesting chlorophyll *a*/*b* · protein (LHCP) of spinach [7]. The amino acids are arranged into hydrophilic, intermediate and hydrophobic groups [35] reading down the table. The values for threonine and serine were obtained by linear extrapolation to zero time after hydrolysis for 24 and 72 h.

	Spinach LHCP [7]	67 000-dalton chlorophyll · protein	21 500--dalton subunit	23 000--dalton subunit	125 000-dalton chlorophyll · protein
Asx	9.3	9.3	9.3	9.1	8.1
Glx	9.2	9.6	8.5	9.3	6.7
His	1.2	1.1	0.7	1.1	4.0
Lys	5.8	4.2	4.7	4.6	7.8
Arg	3.0	3.3	3.0	3.2	6.4
Thr	5.3	4.6	4.4	4.5	5.1
Ser	4.3	6.2	6.0	5.8	7.4
Pro	7.4	6.4	7.0	7.3	3.8
Ala	10.6	9.6	9.0	9.6	9.3
Cys	0.5	—	—	—	—
Gly	13.0	15.7	16.0	15.6	9.8
Tyr	2.6	3.5	3.9	4.2	2.9
Val	6.7	5.7	6.0	5.3	6.7
Ile	4.5	4.6	4.6	4.5	5.7
Leu	9.8	9.3	9.5	9.1	9.7
Phe	5.8	5.7	6.0	5.7	6.2
Met	1.6	1.3	1.8	1.4	1.1

TABLE II

The carbohydrate content of the two isolated subunits of the 67000-dalton chlorophyll *a*/*b* · protein complex of *A. mediterranea*. The sugars were determined as described under Methods.

Sugar	21 500-dalton subunit %	23 000-dalton subunit %
Glucose	0.80	0.70
Galactose	0.09	0.10
Mannose	0.07	0.07
Xylose (?)	0.13	0.13
Ribose (?)	0.03	0.04
Total %	1.12	1.04

the 67 000-dalton chlorophyll *a*/*b* · protein complex from *A. mediterranea*, none of the two polypeptides gave a visible reaction after carbohydrate staining of the gel [27]. However, using the more sensitive method of gas/liquid chromatography for the detection of sugars, it was found that approximately 1 % of each of the two subunits consisted of carbohydrates. As shown in Table II, the sugar composition of both subunits is almost identical.

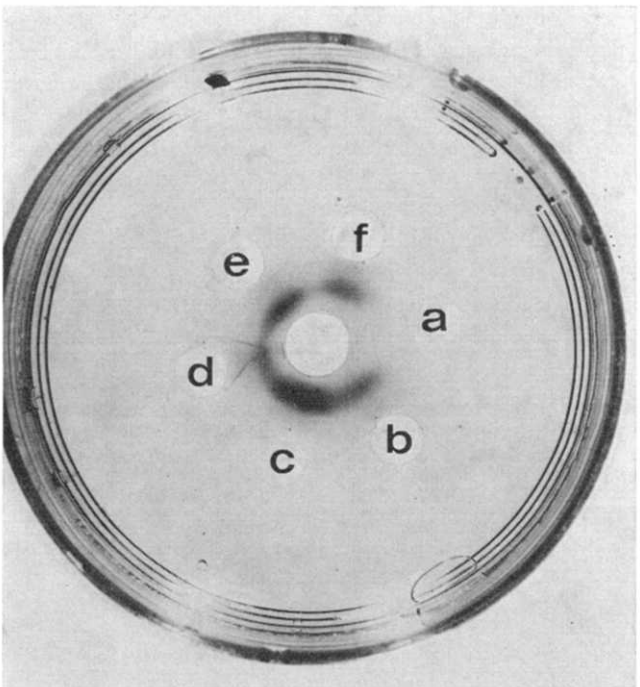

Fig. 3. Ouchterlony double diffusion plates of the chlorophyll-binding proteins of *A. mediterranea* against rabbit antisera. The antiserum against the 67 000-dalton chlorophyll *a*/*b* · protein complex in the center well was tested against (a) the 125 000-dalton chlorophyll · protein, (b) the 67 000-dalton chlorophyll *a*/*b* · protein complex, (c) fraction B_{EDTA}, the dansylated subunits of the 67 000-dalton chlorophyll *a*/*b* · protein complex of 21 500 (d) and 23 000 (e) daltons, and a mixture of the two subunits (f) which had not been dansylated.

(*c*) *Immunological characteristics.* Antisera prepared against the acetone-extracted 67 000-dalton chlorophyll *a*/*b* · protein complex of *A. mediterranea* gave precipitation lines on Ouchterlony double diffusion plates with the two isolated dansylated subunits, a mixture of both subunits, which had not been dansylated, and fraction B_{EDTA} (Fig. 3). The precipitation lines observed in all these cases indicated immunochemical identity with no evidence of spurring or crossing over of precipitation lines. All the antigenic sites on the 67 000-dalton chlorophyll *a*/*b* · protein complex were found in both subunits. The antisera did not react with the isolated 125 000-dalton chlorophyll · protein complex of *A. mediterranea* (Fig. 3).

The different mobilities of the two subunits of the 67 000-dalton chlorophyll *a*/*b* · protein complex during dodecyl sulfate polyacrylamide gel electrophoresis should reflect differences in the structure of the two polypeptides. The amino acid and sugar compositions as well as the immunochemical properties, however, did not show any significant difference between the two subunits. On the other hand, cyanogen bromide treatment and digestion with trypsin revealed structural differences which could explain the difference in electrophoretic mobility of the two subunits of the 67 000-dalton chlorophyll *a*/*b* · protein complex.

(*d*) *The tryptic peptides.* Tryptic peptide maps of the isolated 23 000- and 21 500-dalton polypeptides showed 17 and 18 detectable ninhydrin-positive spots, respectively (Fig. 4). The two subunits seem to share 16 common tryptic peptides based on similar electrophoretic and chromatographic mobilities on the silica gel plates. The 23 000-dalton polypeptide contains at least one tryptic peptide which is not found in the tryptic peptide pattern of the 21 500-dalton polypeptide, while the latter differs in at least two tryptic peptides from the 23 000-dalton polypeptide (Fig. 4).

(*e*) *The cyanogen bromide fragments.* In order to prevent unspecific cleavage of the polypeptides, the cyanogen bromide treatment was performed at 2 °C. Under these conditions not all of the polypeptides were digested (Fig. 2D, E). The cyanogen bromide fragments were separated on a sodium dodecylsulfate polyacrylamide gel and still exhibited at least partially the differences in molecular weight of the two polypeptides before the treatment (Fig. 2). Three of the six cyanogen bromide fragments of the 23 000-dalton subunit were not detectable in the cyanogen bromide fragment pattern of the 21 500-dalton polypeptide. Out of the five fragments of the 21 500-dalton polypeptide two seemed to be specific for this polypeptide. Since cyanogen bromide specifically cleaves the methionyl peptide bond [28] the number of fragments obtained from both subunits is higher than expected considering the methionine content of the polypeptides (3.5 and 2.9 residues per 21 500- and 23 000-dalton polypeptide molecule, respectively). It is possible that some of the bands represent either larger fragments which have not been completely digested or reaggregates of smaller fragments.

Comparison between the 67 000-dalton chlorophyll a/b · protein complex of A. mediterranea and the light-harvesting chlorophyll a/b · protein of higher plants

So far, a chlorophyll *a*/*b* · protein complex of 67 000 daltons which is composed of two different subunits has only been described for *Acetabularia.* The question arises whether the occurrence of this 67 000-dalton chlorophyll *a*/*b* · protein complex is a unique feature of *Acetabularia* or whether a similar large chlorophyll *a*/*b* · protein complex may also occur in other plants.

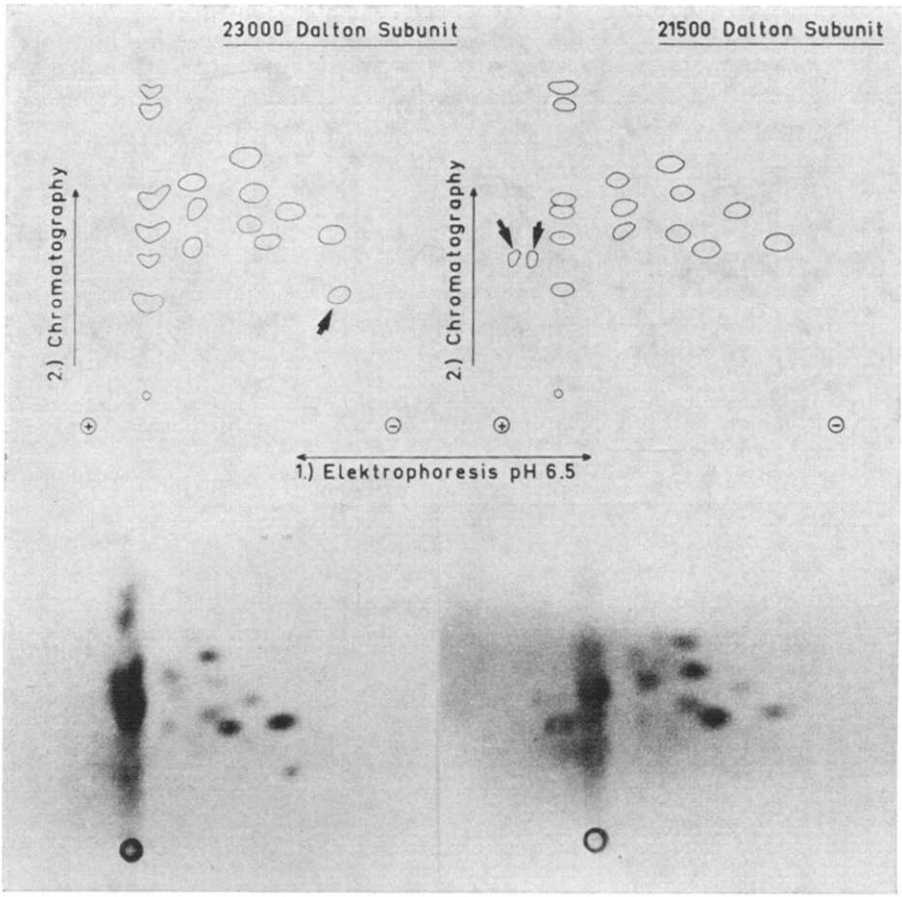

Fig. 4. Photographs and schematic drawings of tryptic peptide maps of the two dansylated subunits of the 67 000-dalton chlorophyll *a*/*b* · protein complex of *A. mediterranea*. Peptide spots specific for either the 23 000- or the 21 500-dalton polypeptides are marked by arrows in the schematic drawing.

Even though there is no proof for the more general occurrence of such a higher-molecular-weight chlorophyll *a*/*b* · protein complex, there is some indirect evidence suggesting that a similar chlorophyll-protein complex may also exist within the thylakoid membrane of other green algae. In *Chlamydomonas* two polypeptides of the thylakoid membranes of 28 000 and 24 000 daltons have been described which seem to correspond to the 21 500- and 23 000-dalton subunits of the 67 000-dalton chlorophyll *a*/*b* · protein complex of *A. mediterranea*. Their amino acid compositions [21] and their immunochemical properties (Chua, personal communication) are almost identical and both polypeptides are found in an isolated light-harvesting chlorophyll *a*/*b* · protein preparation [29].

There have been reports that also in higher plants two polypeptides of approximately 20 000–25 000 daltons exist which may be part of the light-harvesting chlorophyll *a*/*b* · protein complex [30, 31]. However, since in these plants only a light-harvesting chlorophyll *a*/*b* · protein of 27 000–32 000 daltons has been found [7], it

has been concluded that this protein could not contain two polypeptide chains of 20 000–25 000 daltons and it has been suggested that the two polypeptide zones seen in some electrophoretic procedures may be artifactual [7].

The obvious discrepancy between the molecular weights of the 27 000–32 000-dalton light-harvesting chlorophyll *a*/*b* · protein and its two prospective subunits exists only as long as one assumes that this chlorophyll *a*/*b* · protein represents the native entity. The 21 500-dalton chlorophyll *a*/*b* · binding polypeptide of *A. mediterranea* and the light-harvesting chlorophyll *a*/*b* · protein of a higher plant like barley (*Hordeum vulgare*) seem to correspond to each other: The apparent molecular weights are almost identical (Fig. 5) and they have similar amino acid compositions (unpublished results). As shown previously [9], in *Acetabularia* the 21 500-dalton chlorophyll-binding polypeptide is part of the 67 000-dalton chlorophyll *a*/*b* · protein complex. Similarly, also in higher plants two polypeptide chains of 20 000–25 000 daltons might be part of a higher molecular weight light-harvesting chlorophyll *a*/*b* ·

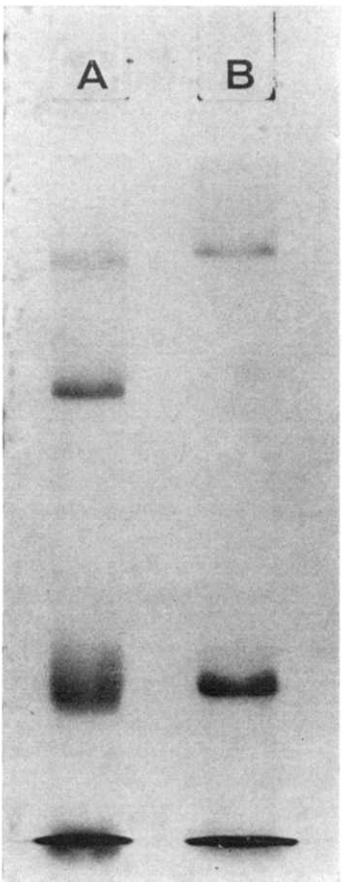

Fig. 5. Comparison of the chlorophyll-binding proteins of *A. mediterranea* (A) and barley (*Hordeum vulgare*) (B). The gel is unstained. The fastest-migrating chlorophyll band contains free chlorophyll while the other bands contain chlorophyll bound to proteins. Electrophoresis was performed at 2 °C in the presence of 0.1 % sodium dodecyl sulfate as described [9].

protein complex. In this case, the chlorophyll · protein of 32000–27000 daltons which is termed "light-harvesting chlorophyll *a*/*b* · protein" could be one of two subunits similar to the 21 500-dalton chlorophyll-binding polypeptide in *Acetabularia*. However, in order to verify this hypothesis two prerequisites must be fullfilled. Firstly, it is necessary to show that in higher plants the two polypeptide chains of 20 000–25 000 daltons are indeed part of the light-harvesting chlorophyll *a*/*b* · protein complex; and secondly, a high-molecular-weight chlorophyll *a*/*b* · protein complex has to be found which contains these two polypeptide subunits.

There have been some reports on the occurrence of higher molecular weight chlorophyll *a*/*b* · proteins in higher plants [32–34]. One of these components, a chlorophyll *a*/*b* · protein complex of approximately 69 000 daltons, seemed to be similar to the 67 000-dalton chlorophyll *a*/*b* · protein complex of *Acetabularia*. Both these complexes were enriched in chlorophyll *b*, were unstable and could be dissociated into a lower-molecular-weight chlorophyll-binding protein. However, the chlorophyll · protein complex described by Hiller et al. [32] was not dissociated into two different polypeptides but only into one chlorophyll-binding protein species of 34 000 daltons. It had been suggested that the larger chlorophyll-protein complex is a dimer of this low-molecular-weight chlorophyll · protein [32]. If in higher plants this 69 000-dalton chlorophyll *a*/*b* · protein complex is composed of two polypeptides which are not only identical in their apparent molecular weight, as indicated by the work of Hiller et al. [32], but also in their primary structure, this would reveal a significant difference in the organization of the light-harvesting chlorophyll *a*/*b* · protein complex of green algae and higher plants.

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