MONOCLONAL ANTIBODIES USED FOR THE CHARACTERIZATION OF THE TWO PUTATIVE IRON-SULPHUR CENTRE PROTEINS ASSOCIATED WITH PHOTOSYSTEM I

by

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Monoclonal antibodies have been produced to the two putative 18.3 and 15.2 kD iron-sulphur centre proteins in barley. When photosystem I particles, containing only chlorophyll *a*-protein 1 and the 18.3 and 15.2 kD proteins, were used as the antigen, thirteen independent clones secreting either IgG or IgM antibodies against the 15.2 kD protein were formed. No hybridomas secreting antibodies to the 18.3 kD protein were detected. When a delipidated polypeptide preparation was the immunogen, we obtained one hybridoma, secreting antibodies of the IgM class to the 18.3 kD protein.

These antibodies were used in immune-blot assays. Neither the 18.3 nor 15.2 kD putative iron-sulphur centre proteins were detected in etioplast membranes. However, in the chloroplast membranes of the photosystem I mutant *viridis-zb*⁶⁴ we found small amounts of these proteins.

The two proteins were purified by electroelution and then tested by immune-blot assay. Fractions with positive reaction were collected and the amino acid compositions determined. There were significant differences in the amounts of serine, glycine and histidine between the two proteins.

1. INTRODUCTION

By comparing electrophoretograms of wild-type barley thylakoid polypeptides with those of mutants with impaired photosystem I (PSI)

activity, four polypeptides are found to be deficient or lacking in these mutants (13, 29, 31). The apparent molecular weights of these polypeptides are 110, 18.3, 15.2 and 14.8 kD. The

Abbreviations: BSA = bovine serum albumin; Chl_a-P1 = chlorophyll *a*-protein 1; C/M PBS-wash = the four polypeptides released from chloroform/methanol extracted thylakoids with PBS; DMEM = Dulbecco's modified Eagles' medium; DTT = dithiothreitol; ELISA = enzyme-linked immuno-adsorbent assay; EPR = electron paramagnetic resonance; Fe-S = iron-sulphur; Ig = immunoglobulin; kD = kilodalton; NBC = newborn calf serum; PAGE = polyacrylamide gel electrophoresis; PBS = phosphate buffered saline; PSI = photosystem I; SDS = sodium dodecyl sulphate; Tris = tris-(hydroxymethyl)-aminomethane; WT = wild-type.

110 kD polypeptide is the chlorophyll *a*-protein 1 containing the reaction centre (*P700*) of PSI (14, 28). The chlorophyll *a*-protein 1 and the 18.3 and 15.2 kD polypeptides are also the constituents of a PSI particle capable of PSI electron transport (29). It has been proposed that the 18.3 and 15.2 kD polypeptides carry two of the Fe-S centre on the reducing side of PSI (29). This has been inferred from *in vivo* labelling experiments with the radioisotope ^{59}Fe . Most of the radioactivity was incorporated into cytochrome *f*, and the rest of the label was found in chlorophyll *a*-protein 1 and two polypeptides with electrophoretic mobilities similar to those of the two low molecular weight polypeptides of the PSI particle (26, 29). While it is clear that these polypeptides are involved in PSI activity (30), it is not certain that both polypeptides contain Fe-S centers. It has been suggested that centers A and B are both located on the same polypeptide (10). Recently a more complex PSI particle from spinach consisting of 5 major polypeptides (110, 20, 17, 16.5, 10 kD) has been analysed for identification of Fe-S centres (24). Upon *in vivo* labelling with ^{35}S the 110, 20, 17 and 16.5 kD polypeptides showed radioactivity, as did peptides with an apparent molecular weight of 8 kD. The 20 and 17 kD polypeptides are likely to be the same as the 18.3 and 15.2 kD polypeptides from barley. LAGOUTTE, SETIF and DURANTON (24) suggest the Fe-S proteins to be among the 8 kD polypeptides.

The barley PSI particle has been studied by electron paramagnetic resonance spectrometry (EPR) (30). These studies revealed that the PSI particle carries the reaction centre chlorophyll *P700* and the four electron acceptors A_1 , X, B and A. The A, B and X signals come from membrane bound Fe-S sulphur centres and A_1 might be a pheophytin molecule or a special chlorophyll radical (7, 12). The EPR signals *P700*, A_1 , X, B and A were all absent from the PSI mutant *viridis-zb*⁶³ (30).

The identification and characterization of polypeptides can also be achieved with the aid of specific antibodies (20). We report here the establishment of hybridoma lines secreting monoclonal antibodies to the two putative Fe-S centre proteins. With the aid of these monoclonal antibodies, using the immune-blotting

technique, it has been possible to further characterize the two polypeptides of apparent molecular weight 18.3 and 15.2 kD.

2. MATERIALS AND METHODS

2.1. Plant material

Seeds of wild-type barley (*Hordeum vulgare* cv. Svalöf's Bonus) and the recessive nuclear gene mutant *viridis-zb*⁶³ were germinated in tap water moistened vermiculite. They were grown in the dark or in the light (1700 lux) at 23 °C. In all experiments the seedlings were harvested seven days after sowing. The mutant seedlings were separated from the wild-type phenotype by their paler green colour.

2.2. Isolation of PSI particles, Fe-S centre proteins, protoplasts and purified thylakoids

Photosystem I particles were isolated using the zwitterionic detergent Empigen BB (29). The polypeptides of apparent molecular weight 32,000, 23,000, 18,300 and 10,000 were extracted from thylakoid membranes with chloroform:methanol (2:1 v:v), followed by a treatment with phosphate buffered saline pH 7.2 (C/M PBS-wash) (15). The two putative Fe-S centre proteins were obtained by electroelution (19). Protoplasts from wild-type and *viridis-zb*⁶³ leaf slices were prepared according to published procedures (6). Internal plastid membranes were purified by flotation in a discontinuous sucrose gradient (21). If the membranes were not used immediately they were frozen in small aliquots in 0.1 M- Na_2CO_3 -0.1 M-DTT at -20 °C. Chlorophyll was determined according to ARNON (1) and protein according to LOWRY (25). Amino acid analysis was carried out after hydrolysis of the protein in 5.7 N-HCl at 110 °C for 24 hours in evacuated sealed tubes.

2.3. SDS-PAGE and electrophoretic blotting procedure

Electrophoresis in SDS was performed as described by CHUA and BENNOUN (4). Immediately after completion of the electrophoretic run the polypeptides were transferred from the SDS-

gel to nitrocellulose filters (Millipore HAHY pore size 0.45 μm) using 125 mM-Tris, 192 mM-glycine pH 8.9, 20% (v/v) methanol as the transfer buffer (17, 37). The electrotransfer was performed for 2.5 hours at 2 A and 60 V in a chromatography chamber with electrodes of acid-hardened stainless steel. The chromatography chamber was placed in ice during the electrotransfer. Immediately after transfer the nitrocellulose filters were cut into strips and kept in the dark at room temperature until used for immune-blot assays. Reference strips were stained with Amido black (0.1% in 45% methanol/10% acetic acid) and destained in methanol/water/acetic acid (2v/2v/0.4v). SDS-gels were stained with Coomassie blue. For calibration of molecular weight and transfer efficiency the following proteins were used: catalase, ovalbumin, chymotrypsinogen A, cytochrome C (Combithek, Boehringer Mannheim).

2.4. Immune-blot assay (37)

The nitrocellulose filter strips with the immobilized antigens were soaked for one hour at 37 °C in 10% newborn calf serum (NBC) in phosphate buffered saline (PBS) pH 7.2. They were then washed with three changes of PBS and incubated for 1-2 hours at 37 °C with hybridoma supernatant or purified monoclonal antibody (40-50 $\mu\text{g}/\text{ml}$) in 10% NBC in PBS. After washing five times in 1% bovine serum albumin (BSA) in PBS, the strips were incubated for 1-2 hours with peroxidase-conjugated rabbit immunoglobulins to mouse immunoglobulins (Dako immunoglobulins a/s cat. no. P161). The secondary antibody was diluted 500-fold with 10% NBC in PBS. The strips were washed five times with PBS before the colour was developed with the aid of o-dianisidine as described in (37) or with 3-amino-9-ethylcarbazole (11).

2.5. Enzyme-linked immunoadsorbent assay (ELISA).

Sterile round-bottom microtestplates (Nunc cat. no. 252162) were incubated for 2 hours with 0.5 μg protein per well of thylakoid membranes or PSI particles. The assay was carried out as described previously (16).

2.6. Immunization

Female mice (Balb/c strain, Bomholtgaard, Ry, Denmark) were injected intraperitoneally with PSI particles or C/M PBS-wash. Two immunization schedules were used (Table I). The first injection was given when the mice were 5-6 weeks old.

2.7. Myeloma line

The two myeloma lines used, X63-Ag8.6.5.3 (22) (Fusion A) and Sp2/0-Ag 14 (Fusion B) (33), were obtained from Dr. G. KÖHLER. These lines do not produce any myeloma globulin chains. The myeloma lines and all the hybridoma lines (beginning 1 month after fusion) were grown in Dulbecco's modified Eagles' Medium (DMEM) supplemented with 1 mM-sodium pyruvate, 100 units penicillin-streptomycin/ml, 2 mM-glutamine, 0.05 mM-2-mercaptoethanol and 10% (v/v) fetal calf serum. This medium is referred to as complete DMEM.

2.8. Fusion

Fusion A (Table I) was carried out as described by FAZEKAS DE ST.GROTH and SCHEIDEGGER (8) plating the fused cells out in microtestplates (Nunc, cat. no. 167008). In fusion B the procedure was slightly modified by increasing the myeloma and spleen cell number by three times to 3×10^8 myeloma cells and 1.8×10^8 spleen cells. During fusion 6 ml of 50% (w/v) polyethylene glycol 4000 (Merck, cat. no. 9727) in 10 mM-sodium phosphate, 137 mM-NaCl, 5 mM-KCl, 10 mM-glucose, 10 mg phenol red was added. However, the fused cells were resuspended in the same final volume of HAT-media (complete DMEM supplemented with 0.1 mM-hypoxanthine, 4×10^{-7} M-aminopterin, 1.6×10^{-5} M-thymidine) before plating out as in fusion A (48 mls). This means that the cell concentration in each well was three times greater in fusion B compared to fusion A.

The hybridoma clones formed were initially screened for antibody production using the ELISA assay with thylakoid membranes as antigens. Positive clones were transferred to 24 well Multidishes (Nunc, cat. no. 143982) and aminopterin was omitted from the media. When the

cells showed confluent growth in the Multidish, the hybridoma clones were tested for antibody specificity by immune-blot assay. The antigens were either total thylakoid membrane polypeptides or PSI particle polypeptides. Cells secreting the desired antibodies were cloned by limited dilution (32) in microtest plates employing 10^4 macrophages per well. The selected clones were grown in complete DMEM in 260 ml tissue culture flasks (Nunc, cat. no. 153732) and the hybridoma supernatant was collected and used directly for assays or pooled and frozen for later use.

2.9. Ig class determination and purification of antibodies

The class of the secreted antibodies was determined initially by an Ouchterlony double diffusion test followed by biosynthetic labelling with ^{35}S -methionine as described earlier (16).

Monoclonal antibodies of the IgG subclasses were purified by chromatography on Protein A-Sepharose CL-4B (Pharmacia). Monoclonal antibodies of the IgM class were purified by ammonium sulphate precipitation followed by chromatography on a Sephacryl S-300 (Pharmacia) column (16).

3. RESULTS

3.1. Fusion frequency and antibody response

By immunizing the mice seven times, instead of three, prior to fusion (Table I) and increasing the cell number 3-fold, the fusion frequency was raised considerably. As judged by the number of wells containing growing hybridomas, it was raised from approximately 50% to 100%. Contamination of some of the initial hybridomas in fusion A with bacteria did not allow us to detect more than a few antibody producing clones from this fusion. However, as judged by ELISA, 6.5% of the clones resulting from fusion B reacted with thylakoid membrane polypeptides. Of these initial clones, thirteen secreted antibodies to the 15.2 kD putative Fe-S centre protein, twelve produced antibodies to the chlorophyll *a*-protein 1 (17) and other lines secreted antibodies to polypeptides in the 30-35 kD range. No hybridoma from fusion B, where PSI particles were the immunogen, produced antibodies to the 18.3 kD protein.

Only in fusion A, where the antigen was obtained from chloroform/methanol extracted thylakoids, did we score one hybridoma that secreted antibodies to the 18.3 kD putative Fe-S centre protein. This clone produced antibodies of the IgM class as determined by the Ouchterlony double diffusion test and biosynthetic labelling. Two hybridomas producing antibodies to the 15.2 kD Fe-S centre protein were em-

Table 1.
Immunization schedules

Injections of thylakoid polypeptides from C/M PBS-wash prior to fusion A		Injections of PSI particles prior to fusion B	
100 µg in FCA	Day 0	84 µg in FCA	Day 0
100 µg in FIA	Day 21	84 µg in FIA	Day 18
100 µg in FIA	Day 42	17 µg in FIA	Day 39
Tail-bleeding	Day 44	Tail-bleeding	Day 46
Fusion	Day 45	17 µg in PBS	Day 50
		168 µg in PBS	Day 53
		336 µg in EB	Day 54
		252 µg in EB	Day 56
		Fusion	Day 57

FCA: Freund's complete adjuvant, FIA: Freund's incomplete adjuvant

EB: elution buffer, 10 mM-Tris-HCl pH 7.6, 200 mM-NaCl, 0.1% Triton X-100

Figure 1. Presence of the putative Fe-S centre proteins in barley chloroplasts and etioplasts.

Chloroplasts (20 µg chlorophyll ~ 100 µg protein) and etioplast membranes (370 µg protein) were separated on 11-15% SDS-PAGE. The separated polypeptides were transferred to nitrocellulose filter and then probed with monoclonal antibodies to the 15.2 and 18.3 kD proteins. Lanes 1 and 2: chloroplast and etioplast membrane polypeptides prior to transfer onto nitrocellulose filter. Lanes 3 and 4: protein left over in the SDS-gel after electrotransfer. Nitrocellulose filters with thylakoid polypeptides (lane 5) and etioplast polypeptides (lane 6) probed with the monoclonal antibody to the 18.3 kD putative Fe-S centre protein, lane 7: etioplast polypeptides and lane 8: chloroplast membrane polypeptides probed with a monoclonal antibody to the 15.2 kD putative Fe-S centre protein.

Figure 2. Test for the presence of the putative Fe-S centre proteins in thylakoids from wild-type and *viridis-zb⁶³*.

Lane 1: wild-type thylakoid polypeptides (20 µg chlorophyll) and lane 2: *viridis-zb⁶³* thylakoid polypeptides (20 µg chlorophyll) separated on 11-15% SDS-PAGE. The separated polypeptides were transferred to nitrocellulose filters which were used in immune-blot assays. Lane 3: wild-type thylakoid polypeptides and lane 4: *viridis-zb⁶³* probed with the antibody to the 18.3 kD polypeptide. Lane 5: wild-type and lane 6: *viridis-zb⁶³* thylakoid polypeptides reacted with an antibody to the 15.2 kD protein.

Figure 3. Test for the presence of the putative Fe-S centre proteins in protoplasts from wild-type and *viridis-zb⁶³*.

Lane 1: solubilised polypeptides of purified wild-type thylakoids, lane 2: solubilised polypeptides of wild-type protoplasts and lane 3: polypeptides of protoplasts from *viridis-zb⁶³* were separated on 11-15% SDS-PAGE. The polypeptides were transferred to nitrocellulose filters. Lanes 4, 5, 6 show protein left over in the gel after electrotransfer. Lanes 7, 8, 9: the filter was probed with a monoclonal to the 15.2 kD protein and in lanes 10, 11, 12: the filter was probed with the monoclonal antibody to the 18.3 kD protein.

ployed in this study. One of the lines secreted antibodies of the IgM and other of the IgG class. After initial specificity identification, all three hybridomas were cloned by limited dilution before they were used in immune-blot assays.

3.2. Are the putative Fe-S centre proteins present in etioplasts from wild-type and chloroplasts from wild-type and *viridis-zb⁶³*?

P700 activity has been observed in bean leaves after only 1 hour of greening (2). This

implies a rapid light-induced synthesis of the polypeptides involved in PSI activity, if these polypeptides are not already present in etioplast internal membranes. Figure 1 shows an immune-blot assay where internal membrane polypeptides from chloroplasts and etioplasts were the antigens. When the nitrocellulose filters were probed with the monoclonal antibodies, bands were always observed among chloroplast polypeptides in the positions of the Fe-S centre proteins (Figure 1, lanes 5 and 8). No reaction with an etioplast membrane polypeptide was observed with our monoclonal antibodies (Figure 1, lanes 6 and 7). In Figure 1 approximately 4 times as much etioplast as chloroplast membrane protein was applied to the SDS-gel. Even when only 5 µg of chlorophyll (~ 25 µg of protein) was used, the two polypeptides are easily detected in chloroplast membranes. Thus, the concentration of the 18.3 kD and 15.2 kD putative Fe-S centre proteins in etioplast internal membranes is less than 1/16 of that in chloroplast membranes. This is consistent with the results of BALTIMORE and MALKIN, who were unable to detect the membrane-bound Fe-S centres in barley etioplasts by EPR spectroscopy at 15 K (3).

The nuclear gene mutant *viridis-zb*⁶³ shows a complete absence of PSI activity as measured both by in vitro electron transport reactions (13) and by fluorescence induction kinetics (34). Judged from electrophoretograms the mutant is lacking chlorophyll *a*-protein 1, the 18.3 and 15.2 kD putative Fe-S centre proteins and a polypeptide of apparent molecular weight 14,800 (13). Therefore, it was of interest to establish if it was possible to detect the two proteins in the thylakoids of the mutant by immune-blot assay. In Figure 2 nitrocellulose filters with thylakoid polypeptides from wild-type and *viridis-zb*⁶³ were probed with the monoclonal antibodies to the 18.3 and 15.2 kD proteins. Where polypeptides from *viridis-zb*⁶³ were the antigens, both the 18.3 kD (Figure 2, lane 4) and the 15.2 kD antibody showed a weak reaction with polypeptides of these apparent molecular weights (Figure 2, lane 6). This demonstrates that small amounts of the 18.3 and 15.2 kD putative Fe-S centre protein are present in *viridis-zb*⁶³ thylakoids.

This result could indicate that the two proteins were synthesized but not inserted into the membranes of *viridis-zb*⁶³. If this were the case, these polypeptides might be detected in a higher concentration in whole protoplasts. Protoplasts from wild-type and *viridis-zb*⁶³ were isolated and after separation of their polypeptides on an SDS-gel they were transferred to nitrocellulose filters, which were used in an immune-blot assay (Figure 3). Although both the 18.3 and 15.2 kD proteins were easily detected in wild-type protoplasts (Figure 3, lanes 8 and 11), no reaction was seen when the antigens were *viridis-zb*⁶³ protoplast polypeptides (Figure 3, lanes 9 and 12). Thus, the concentration of the 18.3 and 15.2 kD polypeptides in protoplasts is lower than in purified thylakoids of the mutant. The result, however, does not allow us to decide whether there is synthesis of the putative Fe-S centre proteins followed by a rapid breakdown, or if only very small amounts of these proteins are synthesized in *viridis-zb*⁶³.

3.3. Identification of electroeluted 18.3 and 15.2 kD polypeptides by immune-blot assay

The purification of the two putative Fe-S centre proteins was attempted by electroelution. Thylakoid polypeptides were separated in an 8 mm thick slab gel and electrophoresed into the wells of an electroelution apparatus to be described elsewhere (19). The method is convenient, but might degrade the protein due to the heat evolved at the anode during electroelution. The electrophoretic mobility of the isolated polypeptides can change after such an electroelution, as shown for the α -subunit of the chloroplast coupling factor, CF₁, (19). Therefore, the initial identification on analytical SDS-gels of the two putative Fe-S centre proteins was verified by immune-blot assays.

In Figure 4A an electroeluted polypeptide of an apparent molecular weight of 18.3 kD was the antigen. Only 1.5 µg of protein was applied on the gel (Figure 4A, lane 6). This was electroblotted to nitrocellulose filters. Although not all 1.5 µg of the 18.3 kD polypeptide was transferred (Figure 4A, lane 4) enough was transferred to

Figure 4. Identification of purified putative Fe-S centre proteins.

The two putative iron-sulphur centre proteins were purified by electroelution and then run on 11-15% SDS-PAGE. After transfer to nitrocellulose filters these were probed with the respective monoclonal antibodies. A: Lane 1: purified 18.3 kD polypeptide; lane 2: wild-type thylakoid polypeptides probed with the 18.3 kD polypeptide antibody. Lanes 3, 4, 5: gel after electrotransfer. Lane 6: 1.5 μ g purified 18.3 kD poly-

peptide, and lane 7: thylakoid polypeptides (20 μ g chlorophyll) prior to electrotransfer. B: Lane 1: PSI particle, lane 2: purified 15.2 kD polypeptide, lane 3: wild-type thylakoid polypeptides probed with a monoclonal antibody to the 15.2 putative Fe-S centre protein. Lanes 4, 5, 6: gel after electrotransfer. Lane 7: thylakoid polypeptides (20 μ g chlorophyll), lane 8: 4.5 μ g purified 15.2 kD polypeptide, and lane 9: 40 μ g protein of PSI particles separated on SDS-PAGE prior to electrotransfer.

react with the monoclonal antibody to the 18.3 kD protein. The non-uniform band in Figure 4A, lane 1 is due to an air bubble between the SDS-gel and the nitrocellulose filter.

The purified 15.2 kD polypeptide produced a broad diffuse zone, which was terminated by a sharp band on the SDS-gel (Figure 4B, lane 8) of the same electrophoretic mobility as the 15.2 kD putative Fe-S centre protein in purified PSI particles and thylakoids (Figure 4B, lanes 7 and 9). In this experiment 4.5 μ g of 15.2 kD polypeptide was applied. The diffuse zone of the polypeptide transferred with greater efficiency than the sharp band (Figure 4B, lane 5) as judged from the stainable protein in the gel after electro-blotting. The diffuse zone reacted with the monoclonal antibody to the 15.2 kD protein, while the transferred sharp band and the 15.2 kD protein in thylakoid and PSI particle polypeptide patterns reacted strongly (Figure 4B, lanes 1, 2 and 3). We considered this a definitive identification of the electroeluted putative Fe-S centre proteins, and determined their amino acid composition. Because Tris, in the electroelution buffer, produces a peak in the same position as histidine one ml samples were dialysed against 500 ml glass-distilled water twice, first 2 and then 18 hours before amino acid analysis. It was of interest to compare the two polypeptides for similarities in amino acid composition. Table II shows that there are distinct differences in the amounts of serine, glycine and histidine. Furthermore, the molecular weights of the two putative Fe-S centre proteins are higher, 21.3 kD and 18.2 kD, respectively, when determined from their amino acid composition, than when estimated on the basis of electrophoretic mobility in SDS-PAGE.

Table II.**The amino acid composition of the putative Fe-S centre proteins associated with PSI.**

Residue	21.3 kD Fe-S centre protein No. ¹⁾ /21,3 ²⁾ kD	18.2 kD Fe-S centre protein No. ¹⁾ /18,2 ³⁾ kD
Asx	17	15
Thr	8	10
Ser	19	9
Glx	24	20
Pro	11	13
Gly	31	19
Ala	18	20
Val	12	14
Met	2	2
Ile	9	6
Leu	16	12
Tyr	2	3
Phe	9	6
His	10	2
Lys	9	11
Arg	8	9
Cys	n.d.	n.d.
Trp	n.d.	n.d.
Total amino acids:	205	171

¹⁾ 24 hours hydrolysis. Uncorrected for loss of threonine and serine²⁾ based on 16 leucine per molecule³⁾ based on 12 leucine per molecule

4. DISCUSSION

The introduction of cell fusion for the production of antigen-specific monoclonal antibodies by KÖHLER and MILSTEIN (23) has considerably improved the possibility to obtain specific antibodies to a wide variety of antigens. The method has proven especially successful for surface antigens of cells and viruses, whereas antibodies to soluble antigens have been obtained with much greater difficulty (36). Therefore, one would expect that surface exposed antigenic sites on the PSI particle would elicit good antibody response. From experiments with trypsin-treated thylakoids (27), we know that parts of chlorophyll *a*-protein 1 is exposed at the thylakoid surface, since the electrophoretic mobility increases after trypsin-treatment. In fusion B (Table I) twelve independent hybridoma clones secreting antibodies to chlorophyll *a*-protein 1 were isolated (17). Furthermore, since we obtained thirteen different clones pro-

ducing antibodies to the 15.2 kD polypeptide, we conclude that this protein must also have some antigenic sites exposed on the thylakoid surface. In contrast, the 18.3 kD protein did not stimulate an antibody response when it was presented to the mouse in a PSI particle. Only when present in polypeptide preparations where the lipids have been removed, did the 18.3 kD polypeptide elicit an antibody response (15). This indicates that the 18.3 kD protein is buried in the lipid matrix of the thylakoid membrane.

In addition to the polypeptide components of the PSI particle, a 14.8 kD polypeptide is reduced or missing in all PSI mutants of barley (13, 29, 31). In *Chlamydomonas* mutants chlorophyll *a*-protein 1 and a specific set of at least six polypeptides (designated 20, 21, 28, 30, 35 and 37) are affected (9). The electrophoretic mobility and chloroform/methanol solubility of polypeptides 20 and 21 indicate that these polypeptides correspond to the two putative Fe-S

centre proteins in barley (9, 15). With the aid of inhibitors these polypeptides have been shown in *Chlamydomonas* to be synthesized in the cytosol (5). In barley it has been shown by immunoprecipitation with monoclonal IgG's of in vitro translated polypeptides, that the 15.2 kD protein is nuclear coded (HØYER-HANSEN, HØJ and HØNBERG, unpublished). Chlorophyll *a*-protein 1, in contrast, is chloroplast coded (5, 35, 38).

The consistent pleiotropic nature of PSI mutants in barley (13) and in *Chlamydomonas* (9) suggests that the assembly of PSI in the thylakoid membrane requires the presence of all its constituents. Studies with the cold-sensitive barley mutant *tigrina-o*³⁴ confirm this. This mutant has unstable chloroplast ribosomes at the restrictive temperature (18). Thus, polypeptides known from other species to be synthesized on chloroplast ribosomes, are present in reduced amounts if not completely missing. In addition, the amount of the 18.3 and 15.2 kD polypeptides is reduced at the restrictive temperature, and there is a clear increase in the amount of these, as well as chloroplast coded polypeptides, at the permissive temperature (18). In the PSI mutant *viridis-zb*⁶³, which has chloroplast ribosomes, a mutation in a nuclear gene prevents the accumulation of the chlorophyll *a*-protein 1 in the thylakoid membrane.

Interestingly, we were able to detect small amounts of the 15.2 and 18.3 kD polypeptides in the thylakoid membranes of the PSI mutant *viridis-zb*⁶³. With the monoclonal antibodies currently available, no chlorophyll *a*-protein 1 was detected in purified thylakoids of this mutant. To determine if the polypeptide constituents of the PSI particle are transcribed in *viridis-zb*⁶³, immunoprecipitations of polypeptides translated in vitro from RNA isolated from the mutant are needed and such studies are in progress.

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