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A CHLOROPHYLL *b*-LESS MUTANT OF *CHLAMYDOMONAS REINHARDII* LACKING IN THE LIGHT-HARVESTING CHLOROPHYLL *a*/*b*-PROTEIN COMPLEX BUT NOT IN ITS APOPROTEINS

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The mutant *pg 113*, derived from *Chlamydomonas reinhardii*, arg_2^- mt^+ (parent strain), completely lacks chlorophyll (Chl) *b* but is still able to grow under autotrophic conditions. The light-harvesting Chl *a*/*b*-protein complex (LHCP) is absent. This is shown (a) by the lack of the corresponding signal in the CD spectrum of thylakoids and (b) by the absence of the band of the LHCP after electrophoresis of partially solubilized thylakoid membranes on lithium dodecyl sulfate polyacrylamide gels. All the other chlorophyll-protein complexes are present. In spite of the absence of the LHCP, all the polypeptide components of this complex are present in the mutant in the same ratios as in the parent strain, although in slightly reduced amounts. The LHC apoproteins are synthesized, processed and transported into the thylakoid membrane of the mutant. Moreover, the phosphorylation of thylakoid membrane polypeptides, which is related to the regulation of the energy distribution between Photosystem I and II, is the same in the mutant and in the parent strain, indicating that phosphorylation is not dependent on the presence of Chl *b*. Electron micrographs of thin sections of whole cells show that there are stacked regions of thylakoids in both the mutant and the parent strain chloroplasts. However, in the mutant, stacks are located near the chloroplast envelope, while long stretches or sometimes circles of unstacked membranes are found in the interior, mostly around the pyrenoid.

Introduction

Mutants are important tools for correlating individual cell components of an organism with a defined structure or function. The studies on Chl *b*-deficient mutants of higher plants or of green algae which do survive and contain a functional photosynthetic electron-transport chain helped much in the identification of the chlorophyll-protein complex II (CP II) as the light-harvesting Chl *a*/*b*-protein complex (LHCP). This CP II-complex is visible as a green band after gel electrophoresis of SDS-solubilized thylakoid membranes. Since in Chl *b*-deficient mutants CP II cannot be detected, this LHCP is not involved in any direct photosynthetic reaction [1]. Regreening experiments with etiolated plants, however, led to the concept that the LHCP and its apoproteins are the primary membrane constituents which are responsible for cation-mediated excitation energy distribution and grana formation [2–4]. Both these chloroplast membrane processes seem to be influenced also by

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Abbreviations: CP, chlorophyll-protein complex; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Chl, chlorophyll; LHCP, light-harvesting chlorophyll *a*/*b*-protein complex; LDS, lithium dodecyl sulfate.

phosphorylation of membrane proteins, especially of the LHC apoproteins [5,6]. This concept is further favored by the finding that isolated LHCP can form cation-dependent aggregates when incorporated into lipid vesicles [7]. However, some of the Chl *b*-deficient mutants hardly fit into this concept. One of the exceptions seems to be the most intensively studied barley mutant *chlorina f2.* Although in this mutant not only CP II, but also two of the three or four LHC apoproteins [8] are missing and hence cannot be phosphorylated [9,27], the thylakoids are extensively stacked [10]. On the other hand, Picaud et al. [11] found a Chl *b*-less mutant of *Chlamydomonas reinhardii* which has apparently no altered polypeptide pattern as compared to the parent strain, but shows only double layers of thylakoids. Therefore, it is interesting to analyze more Chl *b*-deficient mutants with respect to altered LHC apoproteins, phosphorylation, photosynthetic activity and morphology. By screening for pigment mutants of *C. reinhardii* we selected the new Chl *b*-deficient strain *pg 113.* In the present paper we studied this mutant biochemically and morphologically and found a new stacking arrangement of thylakoids. However, apart from the absence of Chl *b* and CP II, essentially no difference in photosynthetic activity nor in the chemical composition between parent and mutant strain could be observed, showing that light harvesting occurs normally even in the absence of the so-called LHCP.

Materials and Methods

Culture conditions were described previously [12]. All media contained arginine hydrochloride (100 mg/l). The media used for the phosphorylation experiments contained, in addition, sodium acetate (2 g/l). Measurement of chlorophyll was according to the method of Arnon [13].

Mutants were induced by ultraviolet irradiation (7% survival) of the parent strain *C. reinhardii* $arg_2^- mt^+$. Colonies with altered color were selected. Among these the pigment mutant *pg 113* containing no Chl *b* was found.

Circular dichroism was measured in a Jasco spectropolarimeter J-500 A. Isolated thylakoid membranes were further broken to small particles by ultrasonication (Branson sonifier S-75, three 10-s periods, position 1). After centrifugation (150 $\times g$, 4 min) the supernatant was used for the measurements. Photosynthetic O_2 evolution by whole cells was assayed in a Clark-type electrode. The electrode chamber contained about 30 μg of total chlorophyll in 4 ml of an O_2-depleted buffer of 50 mM sodium phosphate (pH 7.6), 5 mM $NaHCO_3$ and 10 mM KCl and was illuminated with different light intensities.

In vivo phosphorylation was done according to the method of Owens and Ohad [14]. The cells were harvested at a concentration of $1.6 \cdot 10^6$ cells per ml (100 $\times g$, 5 min) and resuspended in culture medium to a concentration of $4 \cdot 10^7$ cells per ml. After incubation in the dark for 10 h, they were labelled for half an hour with 21 MBq/ml [^{32}P]orthophosphate (Amersham) either in darkness or in the light at 90 $J \cdot m^{-2} \cdot s^{-1}$. The reaction was stopped by addition of Na_2MoO_4 to a final concentration of 20 mM in order to avoid dephosphorylation [14], and by immediate sedimentation (100 $\times g$, 2 min). The cells were washed twice with 100 mM Tris-HCl (pH 7.6), 5 mM $MgCl_2$ and 20 mM Na_2MoO_4. These labelled cells were either solubilized directly at a concentration of $15 \cdot 10^7$ cells per ml in 100 mM Tris-HCl (pH 7.6), 4% SDS and 1% 2-mercaptoethanol (10 min, 60°C) or fractionated into thylakoids, ribosomes, and soluble proteins. For the fractionation, $6 \cdot 10^7$ cells in 0.1 ml were broken with 0.8-ml glass beads in an Eppendorf tube (3 min, 2800 rpm, 0°C). After filtering off the glass beads the thylakoids were isolated from the homogenate by centrifugation as described earlier [12]. For the electrophoretic analysis of the membrane proteins, pelleted thylakoids corresponding to $6 \cdot 10^7$ cells were solubilized in 0.4 ml of 100 mM Tris-HCl (pH 7.6), 20 mM Na_2MoO_4, 2% SDS and 1% 2-mercaptoethanol (10 min, 60°C). To isolate the ribosomes, the supernatant after cell breakage and the first wash solution of the thylakoids were combined and made up to 25 mM magnesium acetate, 1.25 mM K-EDTA, 200 mM KCl and 14 mM 2-mercaptoethanol. The ribosomes were sedimented in a Ti 80 rotor (65000 rpm, 3 h). From the postribosomal supernatant the soluble proteins were precipitated with 10% trichloroacetic acid (15 min, 0°C). The ribosomal sediment was resuspended in 25 mM magnesium acetate, 1.25 mM

K-EDTA, 25 mM Tris-HCl (pH 7.6) and 25 mM KCl. After addition of 2.5% Triton X-100 the ribosomes were pelleted again through a 40% sucrose cushion in the above buffer in an SW 60 rotor (50000 rpm, 16 h). Before electrophoresis the trichloroacetic acid precipitate and the sedimented ribosomes were solubilized in 100 mM Tris-HCl (pH 7.6), 2% SDS and 1% 2-mercaptoethanol (10 min, 60°C).

The polypeptides were analyzed by SDS electrophoresis on a polyacrylamide gradient gel in a system as described by Laemmli [15]. The polypeptides were stained with Coomassie brilliant blue. Gels with radioactively labelled samples were dried and exposed on an Agfa-Curix X-ray film. To detect the green chlorophyll-protein complexes thylakoid membranes were partially solubilized in 100 mM Tris-HCl (pH 7.6) and 1% LDS and an LDS/Chl weight ratio of 5 (30 min, 0°C) and the samples analyzed by LDS-polyacrylamide gel electrophoresis [16].

To isolate the LHCP as an antigen for immunological experiments, thylakoids prepared as described above were partially solubilized in 100 mM Tris-HCl (pH 7.6) and 1% SDS at an SDS/Chl weight ratio of 5 (30 min, 0°C). After short centrifugation the solubilized material was separated by SDS electrophoresis on a 10% polyacrylamide gel in the system of Laemmli [15]. The green band containing the CP II was cut out. The gel pieces were eluted electrophoretically through a nylon mesh into a dialysis bag and the green samples washed against water by ultrafiltration (Filter BM 100, Berghof) and lyophilized. A rabbit was immunized with the antigen by five subcutaneous injections in intervals of 10 days, each containing 250 μg protein in 0.5 ml of 20 mM sodium phosphate buffer (pH 7.4) and 140 mM NaCl. For the first injection the protein solution was mixed with 0.5 ml complete, and for the following injections with incomplete Freud's adjuvant.

For quantitative determination of specific polypeptides, crossed immunoelectrophoresis as described by Chua and Blomberg [17] was used with the following modification. After the first electrophoresis the polyacrylamide gels were stained with Coomassie brilliant blue as usual. In this form they can be stored at 4°C for several days. Before running the second dimension the region on the gel containing the polypeptides of interest was cut out and the gel pieces were dialyzed for two 10-min periods against water and for two 10-min periods against electrophoresis buffer at room temperature.

For electron microscopy whole cells were embedded in the epoxy resin of Spurr according to the instructions given by the manufacturer (Balzers Union). Thin sections were stained with lead citrate and observed in a Philips 300 electron microscope.

Results

The pigment mutant *pg 113* which has been derived from the parent strain *C. reinhardii* arg_2^- mt^+ is capable of growing under autotrophic conditions. Its Chl *b* content is below the limit of the spectrophotometric detection so that the apparent Chl *a*/*b* ratio is very high (Table I). Furthermore, based on the Chl *a* content, the rate of photosynthetic O_2 evolution of mutant cells is comparable to that of the parent strain. This lack of Chl *b* is not dependent on light intensity during cultivation, in contrast to that found in a maize mutant [18]. Dark-grown cells show the same Chl *a*/*b* ratio.

When solubilized thylakoids are subjected to electrophoresis under not fully dissociating conditions on LDS-polyacrylamide gels, several chlorophyll-protein complexes can be detected as green bands. In our experiments the parent strain gives rise to five green bands (Fig. 1). Besides free

TABLE I

CHLOROPHYLL CONTENT AND PHOTOSYNTHETIC ACTIVITY OF PARENT STRAIN AND THE CHL *b*-LESS MUTANT (*pg 113*)

Chl *a* and Chl *b* content in pg per cell. O_2 evolution in μmol/h per mg of total chlorophyll, at 24°C. Low light: 12 $J \cdot m^{-2} \cdot s^{-1}$; high light, 460 $J \cdot m^{-2} \cdot s^{-1}$. The data are from a representative experiment. ps, parent strain.

	Chl *a*	Chl *b*	Chl *a*/Chl *b*	O_2 evolution	
				Low light	High light
ps	2.4	1.0	2.4	20.8	107.6
pg 113	2.3	< 0.08	> 30	19.3	138.8

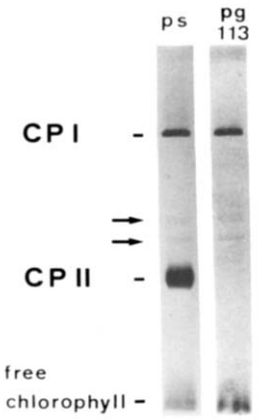

Fig. 1. LDS-polyacrylamide gel electrophoresis of incompletely solubilized thylakoid membranes of parent strain (ps) and the Chl *b*-less mutant (*pg 113*) on a 10% polyacrylamide gel. Gels are not stained, only green bands are visible. Unidentified complexes are labelled with an arrow.

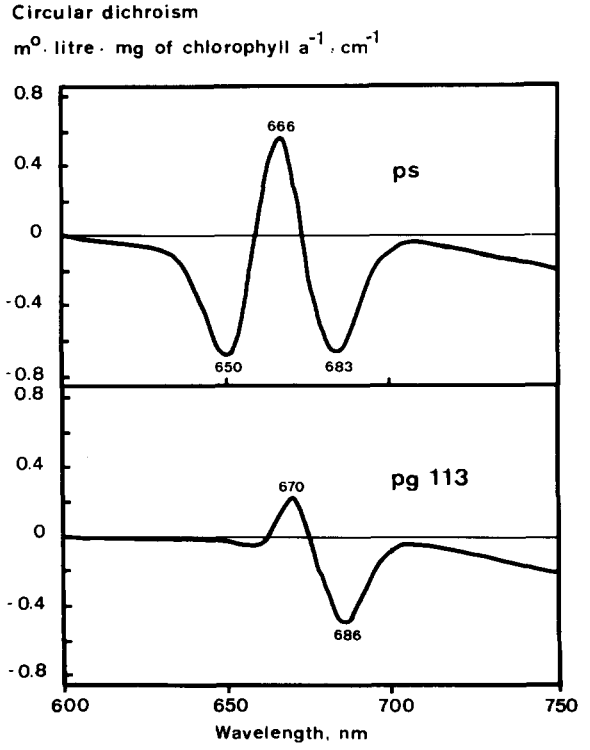

Fig. 2. Circular dichroism of parent strain (ps) and mutant (*pg 113*). The spectra are normalized to the Chl *a* content. The samples contained 0.15 mg/ml (parent strain) and 0.11 mg/ml (mutant *pg 113*) total chlorophyll in 10 mM Hepes-NaOH (pH 6.5). Path length: 1 mm.

chlorophyll, CP I and CP II are the most prominent. In the mutant *pg 113* the CP II band is completely absent while CP I is present at the same intensity as in the parent strain. In both strains two minor bands running between CP II and CP I are also visible. Since these bands are present in the mutant lacking Chl *b* they may tentatively be attributed to the reaction center of Photosystem II rather than to aggregates of CP II.

Circular dichroism of the mutant thylakoid membrane also shows the absence of the signals attributable to CP II [19] when compared with the parent strain (Fig. 2). The characteristic signal of the parent strain with two minima at 683 and 650 nm and a maximum at 666 nm is comparable to published spectra for pea thylakoids [19]. The spectrum can be interpreted as the sum of the

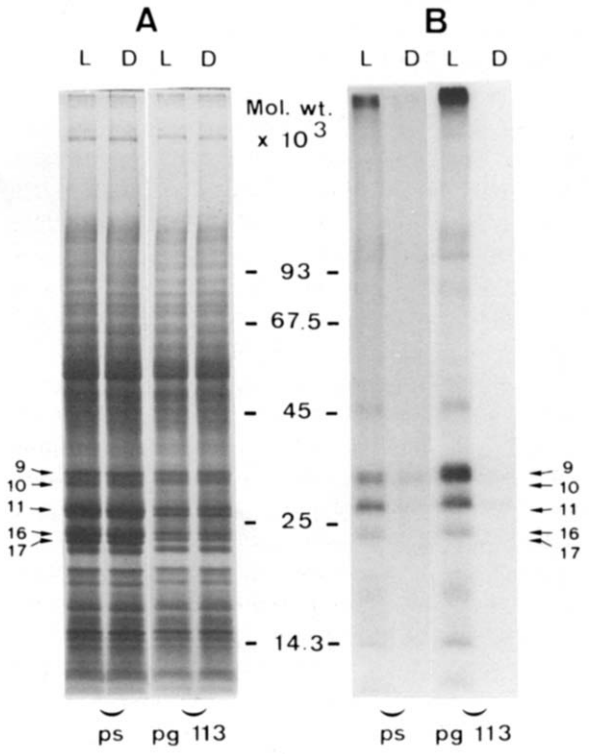

Fig. 3. Electrophoretic analysis of [^{32}P]phosphate-labeled whole cells of the parent strain (ps) and the Chl *b*-less mutant (*pg 113*). After a 10-h dark period cells were labelled with [^{32}P]orthophosphate (30 min) either in the dark (D) or in the light (L), solubilized and electrophoresed on a 7.5–15% polyacrylamide gradient gel of 14 cm length. (A) Coomassie-stained protein pattern. (B) Autoradiograph of the same gel showing the phosphorylated components.

individual signals of isolated CP I and CP II [20]. In the mutant membrane a simple signal can be seen resembling very closely the spectrum of isolated CP I only. The lack of a minimum at 650 nm confirms that the absence of the CP II from LDS-polyacrylamide gels is not an artefact of the solubilization procedure.

The failure to detect CP II by LDS-polyacrylamide gel electrophoresis or by circular dichroism may not necessarily mean that in the mutant no functionally analogous complex exists. In vivo, Chl *a* and the apoproteins could still form a complex which, however, due to the absence of Chl *b*, is extremely sensitive to LDS and shows no signal in circular dichroism. Therefore, it was of interest to look at whether the Chl *b* deficiency was the one and only reason for the absence of CP II or whether other components were also affected by the mutation. Primary candidates would be the apoproteins of CP II, which are believed to be the major substrates for light-dependent phosphorylation.

We incubated exponentially growing cultures initially in the dark for 10 h to dephosphorylate the membrane proteins, and then for 30 min either in the light or dark in the presence of [^{32}P]orthophosphate. The electrophoretic analysis of whole cell proteins shows that all the prominent Coomassie blue-stained polyppetides of the parent strain are also present in the mutant *pg 113* (Fig. 3A). The polypeptides are numbered according to Chua [21], taking into account that polypeptide 16 is completely protected against proteolytic digestion [17,22] and polypeptide 17 is the main phosphorylated polypeptide [14]. The region between 20 and 35 kDa containing the LHC apoproteins shows no difference with respect to the quality of the polypeptides. The autoradiographs of the same gel demonstrate that the phosphorylation pattern is the same in both the parent strain and the mutant *pg 113*. Furthermore, phosphorylation of the polypeptides is dependent on light (Fig. 3B). At least seven prominent bands are radioactively labelled, namely, polypeptides numbered 9, 10, 11 and 17 and three polypeptides with molecular masses of 14, 18 and 45 kDa; in addition, polypeptide 16 is very weakly phosphorylated.

Protein patterns of whole cells have the advantage that artefacts of sample preparation are minimal, but the disadvantage is that they are very complex and indicate nothing about the compartmentation of the individual polypeptides. Therefore, we fractionated ^{32}P-labeled cells of the mutant and of the parent strain into a membrane, a soluble and a ribosomal fraction. Again no noticeable difference in the Coomassie blue-stained patterns (Fig. 4A) can be found between the two strains. The only extra band present in the pattern of the parent strain is some CP I, which originates from the fact that in this particular experiment the Chl-to-SDS ratio was too high in this sample to allow full dissociation of the complex. Otherwise, the close similarity in the patterns indicates that in the mutant the LHC apoproteins are synthesized, processed and transported into the thylakoid membrane as in the parent strain and also that none of these apoproteins occurs in the mutant in a modified, e.g., soluble, form. The autoradiographs, showing the phosphorylated proteins from the same sample, are slightly different between the two strains only in the thylakoid membrane fraction (Fig. 4B). Whereas polypeptides 9, 10, 11 and 17 and the one of molecular mass of 45 kDa are

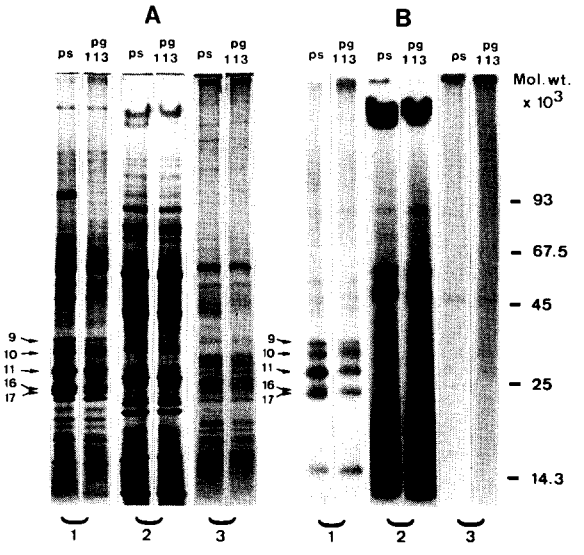

Fig. 4. Fractionation of [^{32}P]phosphate-labelled whole cells into membrane (1), soluble (2) and ribosomal (3) fractions. Samples from the parent strain (ps) and the Chl *b*-less mutant (*pg 113*) were solubilized and separated on a 7.5–15% polyacrylamide gradient gel of 14 cm length. (A) Coomassie-stained protein pattern. (B) Autoradiograph of the same gel showing phosphorylated components.

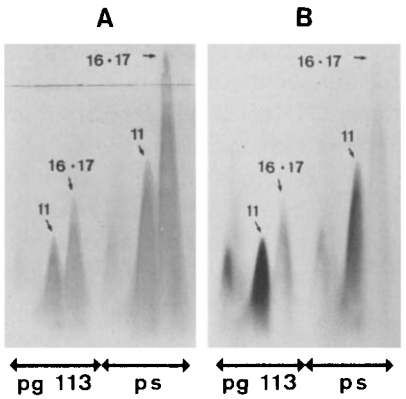

Fig. 5. Crossed immunoelectrophoresis of [^{32}P]phosphate-labelled and then solubilized whole cells of parent strain (ps) and Chl *b*-less mutant (*pg 113*). After the first electrophoresis the Coomassie brilliant blue-stained regions containing the LHC apoproteins were cut out, dialyzed and electrophoresed into the agarose gel containing the antiserum gainst CP II. This agarose gel was dried, stained with Coomassie brilliant blue (A) and exposed to an X-ray film (B). Only light-stimulated phosphorylated samples are presented.

comparably labelled, polypeptide 16 seems to be more labelled in the membrane fraction of the parent strain than in the mutant. We believe that this is a result rather of the isolation procedure than of a real functional difference between mutant and parent strains (compare with the pattern of whole cells in Fig. 3). We have found that isolated thylakoids from the mutant seem to be much less stable during storage than those from the parent strain. This instability has already been described for the barley mutant *chlorina f2* [23].

One might also argue that the absence of CP II in the mutant *pg 113* is due to a reduced amount of the apoproteins. Therefore, we compared quantitatively the content of LHC apoproteins in the two strains by crossed immunoelectrophoresis using a serum raised against the CP II of the parent strain. Fig. 5 shows the analysis of phosphorylated polypeptides of whole cells. Samples of equal numbers of cells were used. All precipitation

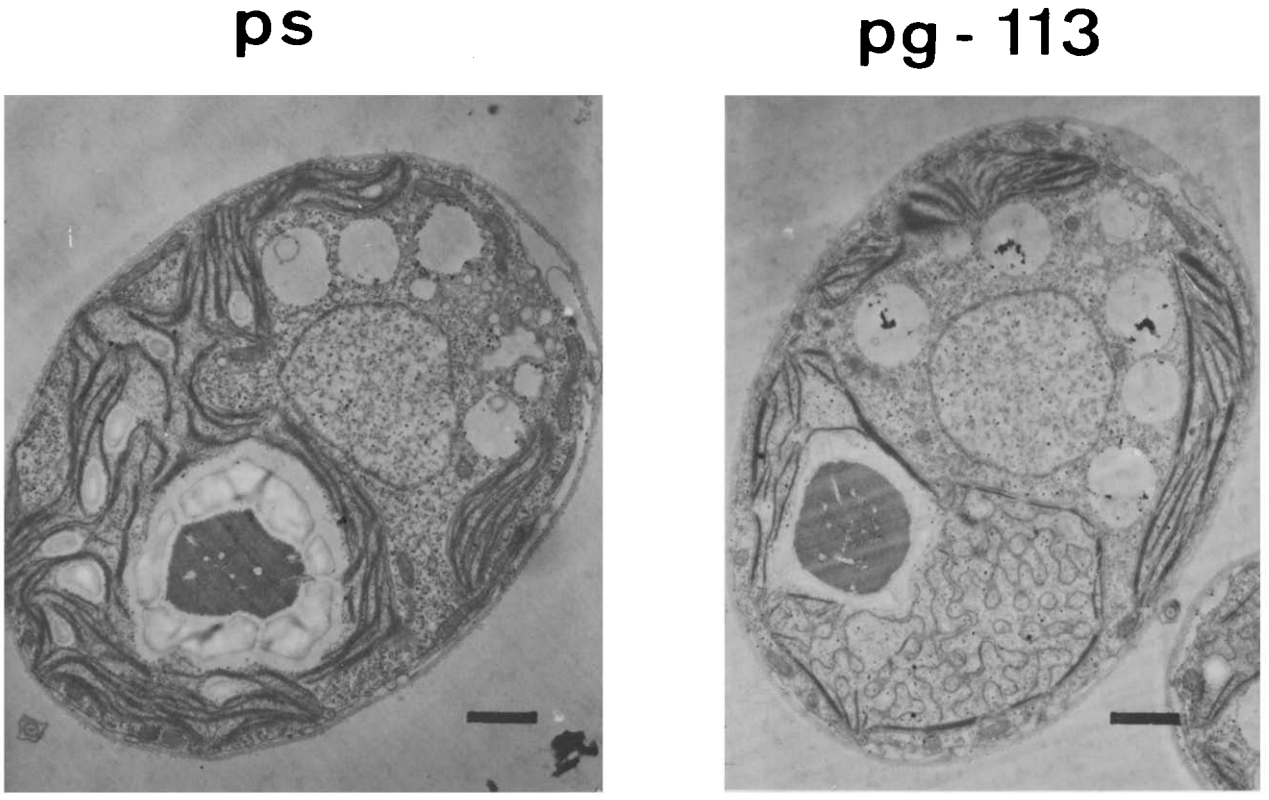

Fig. 6. Electron micrographs of thin sections through whole cells of the parent strain (ps) and the Chl *b*-less mutant (*pg 113*). Bars equal 1 μm.

lines are present in both strains with respect to Coomassie blue staining (Fig. 5A) and to the [^{32}P]phosphate labelling (Fig. 5B). The area under the precipitation lines can be used as a measure of the antigen-to-antibody ratio. From this it can be seen that the amount of polypeptides per cell is reduced in the mutant, but the relative ratios are about the same. Polypeptides 16 and 17 are not resolved under these conditions. However, by cutting out single bands of the first-dimensional polyacrylamide gel and running them separately in the second dimension of crossed immunoelectrophoresis, we were able to confirm that also polypeptides 16 and 17 are present in the same ratios in both strains (data not shown).

There are several indications that the LHCP-PS II complex is involved in stacking [10]. Therefore, it was important to determine whether the stacking of the thylakoids was different between the mutant and the parent strain. On electron micrographs of thin sections of light-grown whole cells (Fig. 6) we found stacked as well as unstacked thylakoids in both strains. However, in the parent strain the stacks were distributed all over the chloroplast, whereas in the mutant, they were concentrated near the envelope and in the 'fingers' of the chloroplast. In the interior of the mutant chloroplast, i.e., around the pyrenoid, an area with long wavy or small circular unstacked double membranes could be observed. Spiral and concentric forms of the membranes found in other mutants of *C. reinhardii* [24] are not seen in our mutant *pg 113*.

Discussion

Chl *b* is associated exclusively with CP II and its oligomeric forms [25]. The monomeric CP II is a green band of 25–30 kDa apparent molecular mass, obtained by SDS-polyacrylamide gel electrophoresis of partially solubilized thylakoids of higher plants or green algae. This CP II band has been identified as the LHCP associated with Photosystem II [10]. Hence, in Chl *b*-deficient mutants this LHCP should somehow be altered. Indeed, in all such mutants the CP II band is absent. However, in contrast to other mutants, e.g., *chlorina f2* of barley [8,9], the relative amounts of the three LHC apoproteins, their phosphorylation and the overall protein composition of the cells are identical to those of the parent strain. The only difference we found between parent strain and mutant, on the molecular level, is the absence of Chl. *b*, and concomitantly of CP II. Interestingly enough, in this mutant *pg 113* the absence of CP II has no obvious effect on the process of light harvesting. With saturating light, the photosynthetic O_2 evolution based on Chl *a* is in the same order of magnitude in the parent and in the mutant strains, indicating that the same number of complete electron-transport chains per Chl *a* is present in both strains. At low light intensities, O_2 evolution and hence quantum yield is very similar in both strains when related to total chlorophyll, showing that although no CP II can be detected by electrophoresis, the Chl *a* in the mutant must be arranged in a light-harvesting device, which is equally effective, but more easily dissociated by LDS than the LHCP of the parent strain. The mutant primarily seems affected on the level of Chl *b* only. All these facts also indicate that Chl *b* is not necessary for the formation of an effective light-harvesting structure in the membrane. However, Chl *b* may be a prerequisite for the formation of a more stable LHCP in the membrane. Indeed, when working with isolated thylakoids one is faced with a more pronounced time- and temperature-dependent loss of Photosystem II activity in the mutant than in the parent strain.

The LHCP has been found to be involved in the stacking of thylakoids, which in turn may affect the energy distribution between Photosystem I and II [2–4,26]. In most Chl *b*-deficient mutants also lacking in CP II, the stacking of thylakoids is different from that in the parent strain. However, the morphological alterations are not the same in the various mutants. In *chlorina f2* of barley, deficient also in two of the LHC apoproteins, thylakoids are intensively stacked [10]. In a Chl *b*-deficient mutant of *C. reinhardii* [11] containing apparently all LHC apoproteins, the thylakoids are arranged in pairs. In our mutant *pg 113* where the LHC apoproteins are also synthesized, processed, integrated into the membrane and even phosphorylated as in the parent strain, regions both with stacked thylakoids and with no stacks at all are found in the chloroplast. From this we conclude that Chl *b* has some effect on the thylakoid arrangement; however, no direct influence of Chl *b* on stacking is obvious.

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References

1 Thornber, J.P. and Highkin, H.R. (1974) Eur. J. Biochem. 41, 109–116
2 Argyroudi-Akoyunoglou, J.H. (1977) in Bioenergetics of Membranes (Packer, L., Papageorgiou, G. and Trebst, A., eds.), pp. 85–96, Elsevier/North-Holland, New York
3 Armond, P.A., Arntzen, C.J., Briantais, J.M. and Vernotte, C. (1976) Arch. Biochem. Biophys. 175, 54–63
4 Davis, D.J., Armond, P.A., Gross, E.L. and Arntzen, C.J. (1976) Arch. Biochem. Biophys. 175, 64–70
5 Barber, J. (1982) Annu. Rev. Plant Physiol. 33, 261–295
6 Bennett, J., Steinback, K.E. and Arntzen, C.J. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 5253–5257
7 McDonnell, A. and Staehelin, L.A. (1980) J. Cell Biol. 84, 40–56
8 Bellemare, G., Bartlett, S.G. and Chua, N.H. (1982) J. Biol. Chem. 257, 7762–7767
9 Haworth, P., Kyle, D.J. and Arntzen, C.J. (1982) Arch. Biochem. Biophys. 218, 199–206
10 Miller, K.R., Miller, J.G. and McIntyre, K.R. (1976) J. Cell Biol. 71, 624–638
11 Picaud, A., Dubertret, G., Guyon, D. and Hervo, G. (1981) in Proceedings of the 5th International Congress on Photosynthesis (Akoyunoglou, G., ed.), pp. 405–415, Balaban International Science Services, Philadelphia
12 Boschetti, A., Sauton-Heiniger, E., Schaffner, J.C. and Eichenberger, W. (1978) Physiol. Plant 44, 134–140
13 Arnon, D.I. (1949) Plant Physiol. 24, 1–15
14 Owens, G.C. and Ohad, F. (1982) J. Cell Biol. 93, 712–718
15 Laemmli, U.K. (1970) Nature 227, 680–685
16 Delepelaire, P. and Chua, N.-H. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 111–115
17 Chua, N.-H. and Blomberg, F. (1979) J. Biol. Chem. 254, 215–223
18 Miles, C.D., Markwell, J.P. and Thornber, J.P. (1979) Plant. Physiol. 64, 690–694
19 Scott, B. and Gregory, R.P.F. (1975) Biochem. J. 149, 341–347
20 Gregory, R.P.F., Borbély, G., Demeter, S. and Faludi-Daniel, A. (1982) Biochem. J. 202, 25–29
21 Chua, N.-H. (1980) Methods Enzymol. 69c, 434–446
22 Michel, H.P., Schneider, E., Tellenbach, M. and Boschetti, A. (1981) Photosynth. Res. 2, 203–212
23 Boardman, N.K. and Highkin, H.R. (1966) Biochim. Biophys. Acta 126, 189–199
24 Nicholson-Guthrie, C.S., Turner, F.R. and Hudock, G.A. (1975) Exp. Cell Res. 93, 240–244
25 Thornber, J.P., Alberte, R.S., Hunter, F.A. and Kan, K.S. (1977) in Chlorophyll Proteins. Reaction Centers and Photosynthetic Membranes (Olson, J.M. and Hind, G., eds.), pp. 132–148, Brookhaven National Laboratory, Upton, NY
26 Steinback, K.E., Burke, J.J. and Arntzen, C.J. (1979) Arch. Biochem. Biophys. 195, 546–557
27 Ryrie, I.J. (1983) Eur. J. Biochem. 131, 149–155