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# Presence of Polypeptides of Cytoplasmic and Chloroplastic Origin in Isolated Photoactive Preparations of Photosystems I and II in *Chlamydomonas reinhardi* y-1[1]

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## ABSTRACT

**Photoactive preparations were obtained by treatment of photosynthetic membranes with lipid micelles and deoxycholate.**

**A photosystem I preparation can be extracted from the membranes by both lipid micelles and deoxycholate, whereas the photosystem II preparation remains in a particulate state.**

**The photosystem II preparation exhibits electron transfer activity measured by photoreduction of 2,6-dichlorophenolindophenol with 1,5-diphenylcarbazide as an electron donor and has a ratio of chlorophyll *a*/*b* of 1.4. This preparation contains three major polypeptides of cytoplasmic origin and two of chloroplastic origin.**

**The photosystem I preparation exhibits high rates of methylviologen photoreduction with ascorbate and 2,6-dichlorophenolindophenol as electron donors and does not contain chlorophyll *b*. This preparation contains two major polypeptides of chloroplastic origin and one of cytoplasmic origin. These results indicate that both cytoplasm and chloroplast contribute polypeptides for the formation of photosystems I and II in *Chlamydomonas reinhardi*.**

The photosynthetic membranes of *Chlamydomonas reinhardi* grow by addition of proteins of both cytoplasmic and chloroplastic origin to preexisting membranes (5, 7, 16). It has been claimed that membrane polypeptides originating in the chloroplast are required for photosynthetic activity whereas membrane polypeptides of cytoplasmic origin are connected with the appearance of the membrane structure (16).

Fractionation of photosynthetic membranes of *C. reinhardi* y-1 by either digitonin (13) or Triton X-100 (8) results in the formation of two types of particles exhibiting photosynthetic electron transfer activities and spectral properties of either photosystem I or II. Analysis of such particles for their polypeptide composition by gel electrophoresis showed that the PSII-like fraction is enriched in polypeptides of cytoplasmic origin whereas the PSI-like fraction is slightly enriched in membrane polypeptides originating in the chloroplast (8, 13). However, mutant strains of *C. reinhardi* lacking certain membrane polypeptides of cytoplasmic origin exhibit normal PSII activity (12).

To identify the polypeptides required for the formation and activity of both photosystems, as well as the distribution of polypeptides of different origins in the isolated photosystems, photosynthetic membranes were fractionated with lipid micelles and deoxycholate, which preserve both PS activities. The preparations so obtained were highly enriched in few, specific polypeptides and exhibited spectral properties and electron transfer activities characteristic of PSI or PSII.

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## MATERIALS AND METHODS

**Cultivation of Cells, Greening, and Degreening Experiments.** *C. reinhardi* y-1 cells were grown on a mineral medium containing acetate as the sole C source as described by Ohad *et al.* (17). Cells were harvested in the logarithmic phase of growth (cell concentration about $2 \times 10^6$ cells/ml) by centrifugation at 40000*g* for 10 min. Degreening (17) and greening (18) were performed as described. For radioactive labeling of membrane polypeptides, $^3$H-Na-acetate was added during the greening process (5).

**Membrane Preparation.** Chloroplast membranes used for assays of photosynthetic activity were obtained by differential centrifugation of homogenates prepared by breaking cells in a French pressure cell, as previously described (20).

For gel electrophoresis analysis and for fractionation of membranes, the homogenate was layered on a linear (8) or a discontinuous sucrose gradient (1.0620 g/cm$^3$ [15%], 8 ml; 1.1315 g/cm$^3$ [30%], 8 ml; 1.2936 g/cm$^3$ [60%], 8 ml) and treated as described (8).

**Gel Electrophoresis.** Polypeptide analysis was carried out by SDS-polyacrylamide gel electrophoresis according to Laemmli (11).

For analysis of the distribution of radioactivity in the gels, frozen gels were cut in 1-mm slices, dissolved overnight in 0.3 ml of 2% (w/v) SDS in $H_2O_2$ at 60 C, and counted in a toluene-Triton scintillation fluid.

**Fractionation of Membranes by Lipid Micelles and Deoxycholate.** Fractions were obtained from membranes by treating them successively with lipid micelles and increasing concentrations of deoxycholate (Fig. 1).

The treatment with lipid micelles was according to a modification of the method described by Toson *et al.* (23).

Lipid micelles were prepared by sonication for 1 min at 4 C with a Branson Sonifier model S 125 at setting 5, of asolectin (commercial total lipid extract of soybean) suspended in a 0.25 M sucrose, 1 mM EDTA, and 10 mM tris-HCl buffer (pH 7.4) solution. The membranes were suspended in the above solution and mixed with the lipid micelles (10 mg lipids/mg protein) as described (23). The mixture was centrifuged for 30 min at 100,000*g* and the pellet obtained was then treated with deoxycholate, using a modification of the method described by MacLennan (15) (Fig. 1).

Supernatant fractions were dialyzed at 4 C against water overnight and then lyophilized.

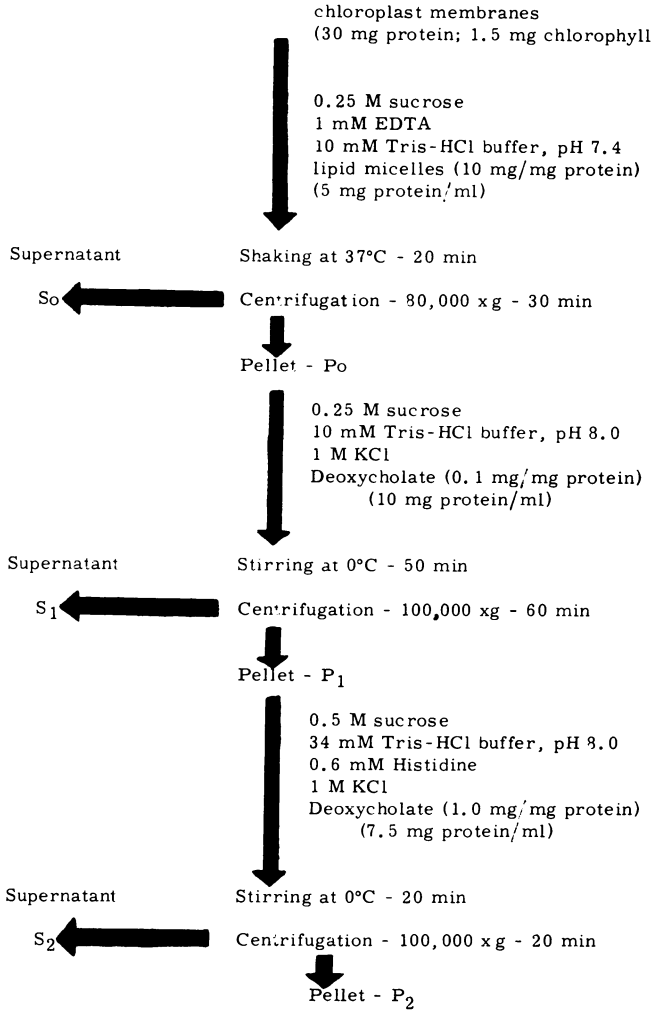


FIG. 1. Procedure for membrane fractionation with lipid micelles and deoxycholate.

**Photosynthetic Activities.** Methylviologen and DCIP[2] photoreduction activities of membranes and membrane fractions were assayed as previously described (20).

Absorption spectra of membranes and of membrane fractions were recorded using the Aminco Chance spectrophotometer operated in the split beam mode.

Chlorophyll was estimated according to the method of Arnon (2) and protein was measured according to Lowry *et al.* (14).

Gels stained with either Amido black or Coomassie brilliant blue, were scanned as described (5). Radioactivity of samples was measured using a Packard model 3380 scintillation counter. Light intensity was measured with a YSI-Kettering model 65A radiometer.

All reagents used throughout this work were of analytical grade.

## RESULTS

**Fractionation of Membranes by Treatment with Lipid Micelles and Deoxycholate.** Lipid micelles readily interacted with the membranes. Mixing of chloroplast membranes with lipid micelles resulted in a decrease in the apparent buoyant density of the membranes from 1.23 g/cm³ to 1.18 g/cm³, indicating inclusion of the lipids into the membranes. When mixing was followed by sonication, the reduction in the membrane density was even greater (1.14 g/cm³). This treatment also extracted high mol wt (>40,000) membrane polypeptides into the lipid micelle fraction, as shown by gel electrophoresis analysis, causing an increase in the lipid micelles' apparent buoyant density from 1.084 g/cm³ to 1.097 g/cm³. The same polypeptides were extracted whether using micelles prepared from asolectin (60% lecithin), total chloroplast membrane lipid extract (6) or purified (1) galactolipids which are characteristic of chloroplast membranes (6).

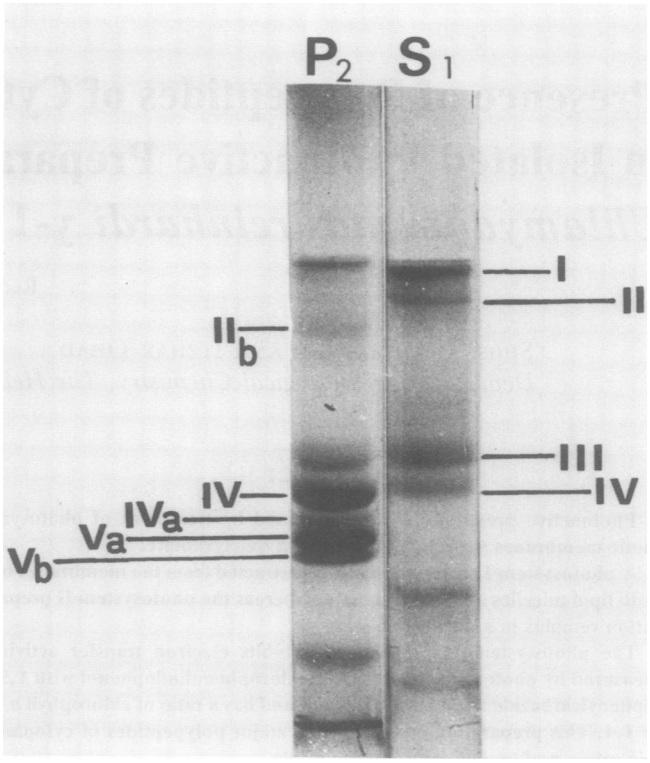


FIG. 2. Polypeptide composition of fractions $P_2$ and $S_1$, exhibiting photosystems II and I activity as shown in Table I. Membranes obtained from $7 \times 10^9$ light-grown cells were fractionated according to Figure 1. Notice the presence of polypeptides I, III, and IV in both preparations. Polypeptides III, IV, Va, and Vb are of cytoplasmic origin (see Fig. 3); polypeptides I, II, IIb, and IVb are of chloroplastic origin (see Fig. 7).

Treatment of membranes with 0.1 mg deoxycholate/mg protein resulted in the extraction of the same high mol wt polypeptides ($S_1$, Fig. 2). When a higher deoxycholate concentration was used (1 mg/mg protein), the soluble fraction contained all membrane polypeptide species. The residual particulate fraction was highly enriched in polypeptides in the mol wt range of 20,000 to 40,000 ($P_2$, Fig. 2). The polypeptide composition of the different fractions was analyzed by SDS-polyacrylamide gel electrophoresis.

It has been previously reported that polypeptides in the 20,000 to 40,000 mol wt range are synthesized by cytoplasmic ribosomes (5, 7). The literature in this area, however, is confusing and the polypeptide pattern reported by different laboratories is not identical (4, 5, 7, 12). Thus, it was necessary to establish the cytoplasmic origin of the various polypeptides resolved in this work. The four major polypeptides in this region are radioactively labeled during membrane formation in presence of chloramphenicol[3] (Fig. 3). Upon fractionation of such

[2] Abbreviations: DCIP: 2,6-dichlorophenolindophenol; DPC: 1,5-diphenylcarbazide.

[3] For comparison, our polypeptides III and IV coincide with polypeptides IIa and IIb, respectively, described by Hoober (7) and others (12, 13). Their single polypeptide IIc was resolved by us into two polypeptides, Va and Vb.

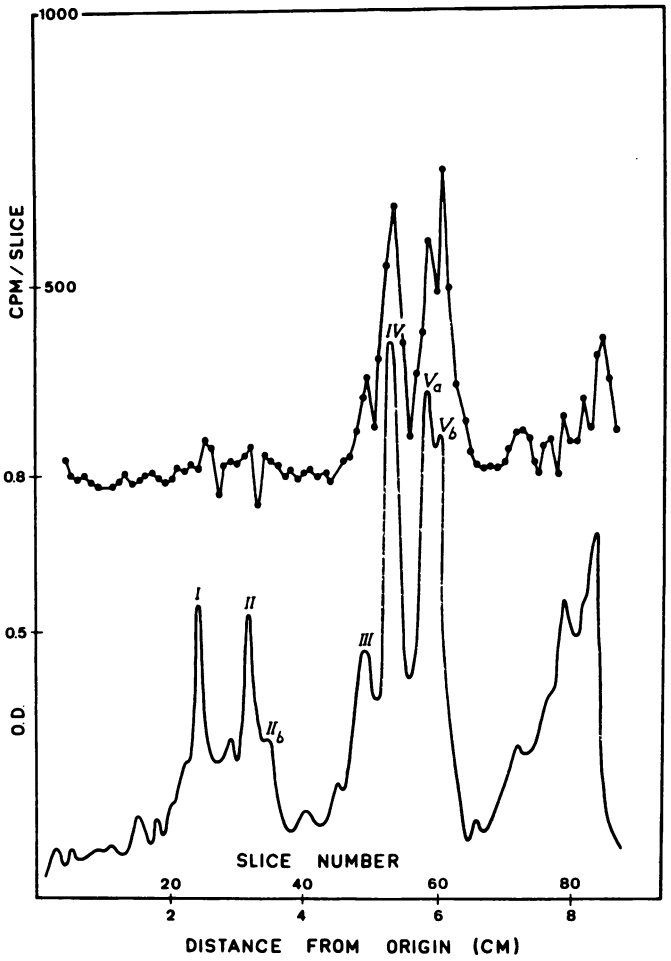


FIG. 3. Identification of membrane polypeptides of cytoplasmic origin by radioactive labeling during greening of y-1 cells in the presence of chloramphenicol. Dark-grown cells were illuminated for 8 hr in the presence of chloramphenicol (200 $\mu$g/ml) and $^3$H-Na-acetate (0.2 Ci/mol, 2 mM). Membranes were prepared and 300 $\mu$g protein per gel (17,000 cpm), were separated by SDS-gel electrophoresis, and analyzed for radioactivity distribution. Recovery of the radioactivity was 85%.

membranes, all of the radioactivity was found in the $P_2$ fraction, migrating with the 20,000 to 40,000 mol wt polypeptides (Fig. 4). Treatment of whole membranes with lipid micelles and deoxycholate did not alter the electrophoretic mobility of any of the polypeptides.

**Photosynthetic Activity of the Isolated Fractions.** The particulate $P_2$ fraction exhibited electron transfer activity typical of photosystem II, whereas the $S_1$ fraction was highly active in photoreduction of methylviologen, typical of photosystem I (Table I). The absorption spectra of the two fractions were also different. The $P_2$ fraction was rich in Chl *b* (Chl *a*/*b* ratio 1.4) and the Chl *a* showed a $\lambda_{max}$ at 677 nm. The $S_1$ fraction did not contain Chl *b* and showed an absorption maximum of Chl *a* at 670 nm (Fig. 5). Fractions $P_2$ and $S_1$ were also prepared from membranes derived from cells greened in the presence of chloramphenicol. These fractions were found to be devoid of any photosynthetic activity, although they contained the polypeptides of cytoplasmic origin as in fractions prepared from active membranes. The minor bands found in the active fractions, but much less evident in the preparation obtained from inactive membranes (Fig. 6), are probably of chloroplastic origin. In order to insure that these minor bands are indeed of chloroplastic origin, cells greened in the presence of chloramphenicol were further incubated and labeled in a "repair" experiment (16) in the presence of cycloheximide. Figure 7 demonstrates that the bands II, IIb and IVa were labeled and correspond with the minor bands specific to the active fractions. In addition, the polypeptides IIb and IVa were enriched in the $P_2$ active preparation, while the polypeptide II was more evident in the $S_1$ active fraction.

## DISCUSSION

The origin of the photosynthetic membrane polypeptides and the role of different polypeptides in the photosynthetic electron transfer has been the subject of intensive investigation (9). It has been accepted that in *C. reinhardi*, several major membrane polypeptides of mol wt ranging between 20,000 and 40,000 are the translation products of cytoplasmic ribosomes (16). The role of polypeptides of cytoplasmic origin in the formation and activity of the photosynthetic membranes has been an open question. These polypeptides were found to be associated with the photosystem II complex, prepared by treatment with digitonin (13) or Triton X-100 (8). The polypeptide composition of PSII complex, prepared with Triton X-100 and analyzed by acidic gel electrophoresis, consisted of mostly L protein(s) (8). When the complex so isolated was analyzed by SDS-gel electrophoresis, it was found to consist of polypeptides III, IV, Va, and Vb, identified as being translated in the cytoplasm (unpublished results). Eytan and Ohad (5) have proposed that the polypeptides made in the cytoplasm are essential for the formation of the membrane structure but not sufficient to allow photosynthetic electron flow which requires also the presence of membrane polypeptides of chloroplastic origin. Photosystem I complex was considered to consist of higher mol wt polypeptides (> 50,000) mostly of chloroplastic origin (8, 13). The chlorophyll-protein I and II complexes, prepared according to Thornber and Highkin (22), have polypeptide compositions similar to those of the PSI and PSII fractions, prepared by digitonin or Triton X-100 (3, 21).

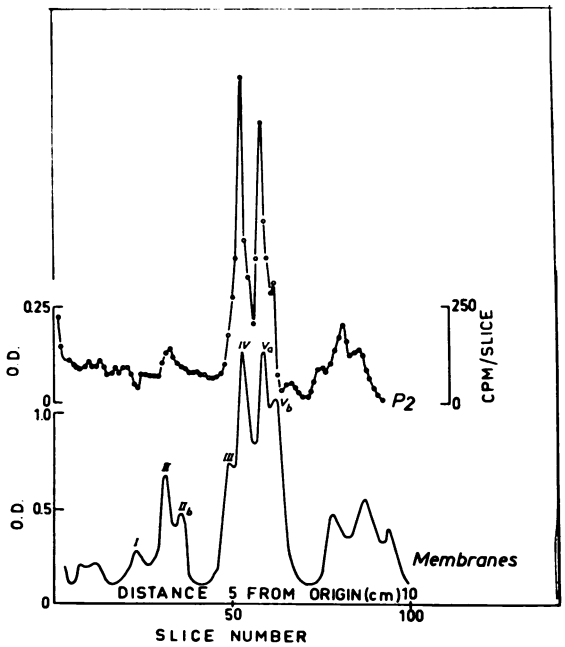


FIG. 4. Migration of the polypeptides of the $P_2$ fraction as membrane polypeptides IV, Va, and Vb, made in the cytoplasm. Membranes, obtained from 2 × $10^{10}$ cells greened in the presence of chloramphenicol (200 $\mu$g/ml) and $^3$H-Na-acetate (1 Ci/mol, 2 mM), were fractionated by the method described in Figure 1. The $P_2$ fraction was run by SDS-gel electrophoresis. Recovery of radioactivity was 80%.

Table I. Photosynthetic Activity of Different Membrane Fractions

Fractions were prepared according to Fig. 1. The control rates (umol substrate·mg $Chl^{-1}$·$hr^{-1}$) were 26 for DCIP with DPC as an electron donor, and 240 for methylviologen.

| Fraction | Ratio Chl to Protein | Chl content | Photoreduction activities | | Ratio II/I | Recovery of activity | |
|---|---|---|---|---|---|---|---|
| | | | Methyl-viologen[2] | DCIP[1] | | | |
| | | | I | II | | I | II |
| | w/w | mg | | | | % | |
| Membranes | 0.04 | 0.9 | 100[3] | 100[3] | 1 | 100 | 100 |
| $S_0$ | 0.04 | 0.45 | 322 | 35 | 0.3 | 112 | 4 |
| $S_1$ | 0.02 | 0.03 | 244 | 50 | 0.2 | 10 | 0.4 |
| $P_2$ | 0.06 | 0.35 | 33 | 857 | 26 | 5 | 77 |
| Recovery(%) | --- | 92 | | | | 127 | 81.4 |

[1] DCIP photoreduction was 90% inhibited by 0.1 mM DCMU

[2] Addition of plastocyanin did not increase methyl viologen photoreduction activity of the membranes and of $S_0$ and $S_1$ fractions, but slightly increased the residual activity found in $P_2$ fraction.

[3] When asolectin and deoxycholate, in concentrations used for fractionation, were added to whole membranes during the activity assay, photoreduction rate of methyl viologen was slightly enhanced, while that of DCIP was reduced to 25%.

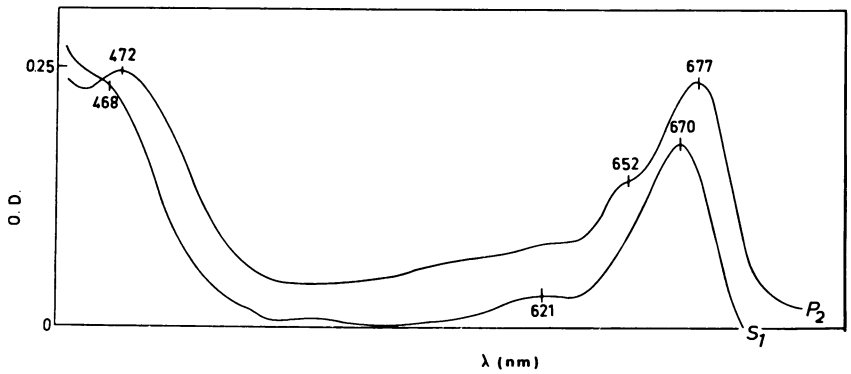


FIG. 5. Absorption spectra of $P_2$ and $S_1$ fractions, prepared as shown in Figure 1. Notice the relative high absorption at 652 nm and maximal absorption at 677 nm of the $P_2$ fraction as compared with only one peak at 670 nm in the $S_1$ fraction.

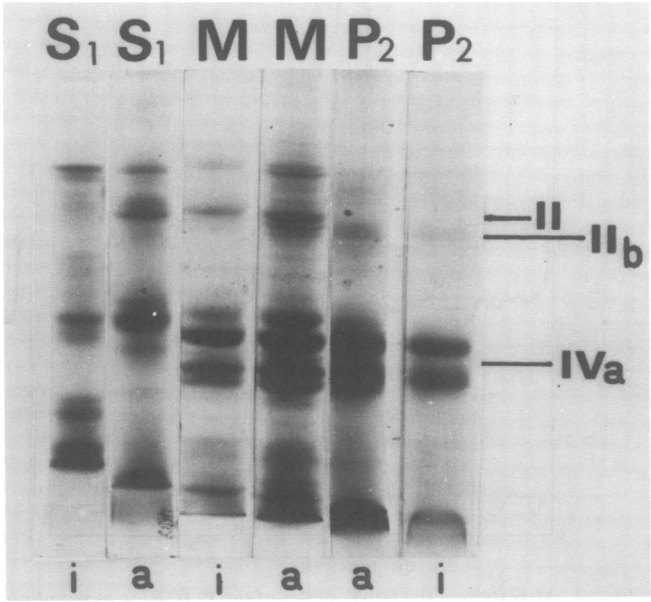


FIG. 6. Electrophoretic pattern of $P_2$ and $S_1$ fractions obtained from active and inactive membranes. Photosynthetically active membranes were obtained from light-grown cells. Inactive membranes were obtained from cells after 10 hr of greening in the presence of chloramphenicol (200 μg/ml). Fractions were obtained in all cases according to Figure 1; a: active fractions; i: inactive fractions; M: whole membranes.

Thus, an apparent discrepancy appears to exist. Photosystem II is considered to consist of polypeptides made in the cytoplasm; however, it is not photochemically active when polypeptides originating in the chloroplast are not present. Additionally, in *C. reinhardi* ac-5 mutant, lacking polypeptides made in the cytoplasm, PSII activity is present (12). During greening, neither synthesis of the chloroplastic polypeptides, which undergo enrichment in PSI particles, nor the development of PSI activity occur in *C. reinhardi* y-1 in absence of concomitant synthesis or preaccumulation of the membrane polypeptides made in the cytoplasm (16). This discrepancy seems to be due to the fact that the data showing uneven distribution of cytoplasmic and chloroplastic membrane polypeptides between the two isolated photosystems were based on analysis of only partially purified fragments (8, 13). On the basis of the data presented in this work, both photosystem I and II photoactive preparations contain polypeptides of chloroplastic and cytoplasmic origins, both of which are essential for their development. Moreover, two of the polypeptides of cytoplasmic origin (III and IV) are common to both preparations. Polypeptide III is apparently not required for PSI or PSII electron flow, since it can be removed by trypsin digestion of the membranes without loss of these activities (19).

It is important to note that both complexes contained only a small number of polypeptides, both were obtained from the same starting material with a high yield and recovery of initial activity, and both were inactive when the polypeptides made in the chloroplast were absent.

Recently it has been shown that, the $T_4$ temperature-sensitive

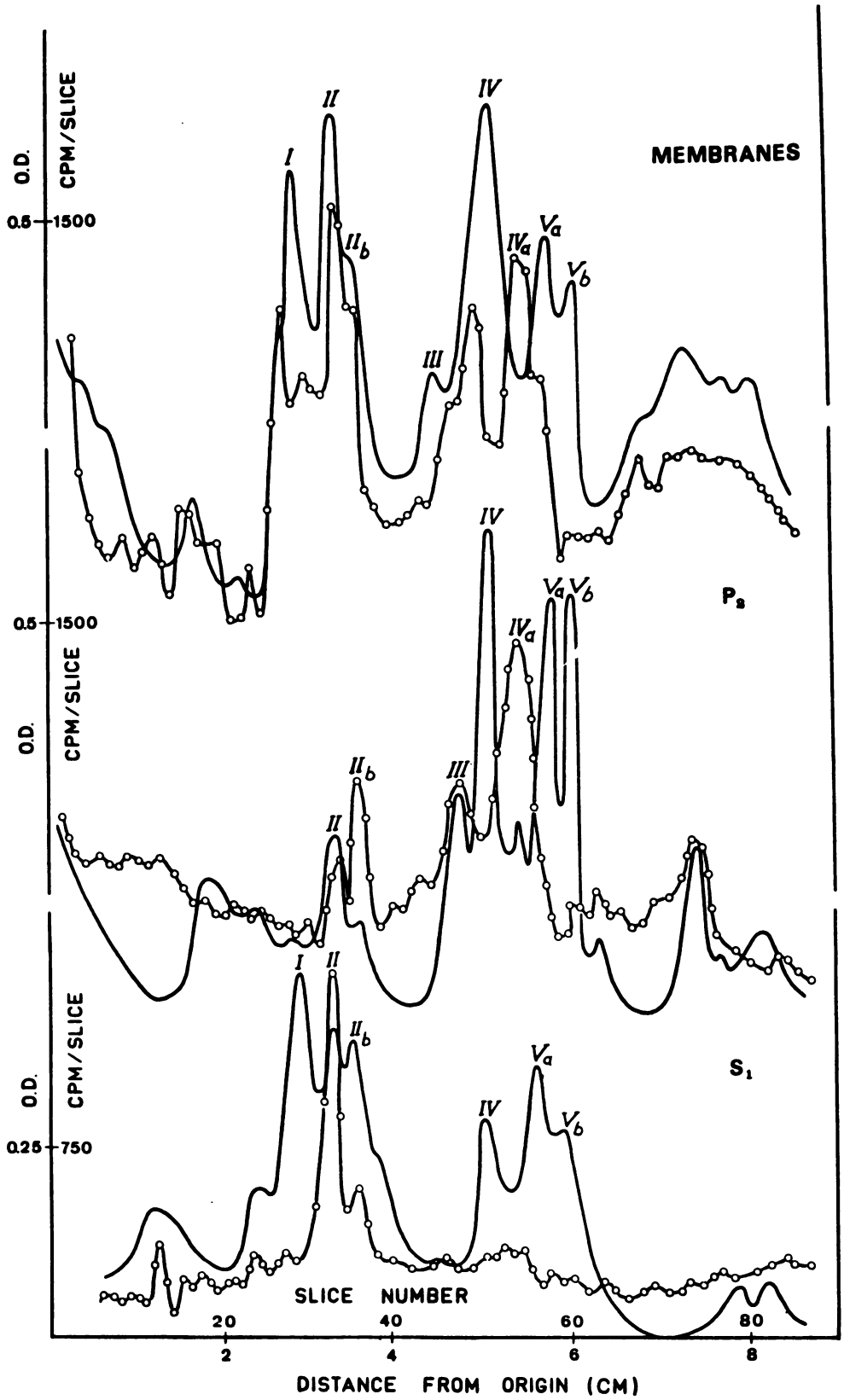


FIG. 7. Polypeptides of chloroplastic origin, in the $P_2$ and $S_1$ fractions, identified by radioactive labeling. Membranes were obtained from cells after greening for 8 hr in the presence of chloramphenicol (200 μg/ml) followed by washing and further incubation for 4 hr in the presence of cycloheximide (0.5 μg/ml) and $^3$H-Na-acetate (2 Ci/mol; 2 mM). Membranes were fractionated according to Figure 1, polypeptides were separated by SDS-gel electrophoresis, and analyzed for radioactivity distribution. Radioactivity recovery was 86%.

mutant of *C. reinhardi* (4) grown at nonpermissive temperature (37 C) lacked two polypeptides of chloroplastic origin in the mol wt range of polypeptide IIb. The PSII activity was absent in this case, while PSI activity was not affected. The PSII activity could be restored following synthesis and insertion into the membrane of the two missing polypeptides at permissive temperature (25 C) (10). It could additionally be postulated that the polypeptides of chloroplastic origin are required for the formation of photosynthetic reaction centers, since other photosynthetic electron transfer components such as Cyt *f*, ferredoxin, and plastocyanin are already present in the nonactive membranes (16).

This would explain the fact that membranes formed in the presence of chloramphenicol and thus lacking polypeptides of chloroplastic origin, are devoid of photosynthetic activities, even when measured with artificial electron donors and acceptors which would enable detection of activities related directly to the active centers. Indeed, during the repair of inactive membranes formed in the presence of chloramphenicol, formation of active centers of PSII was indicated by development of fluorescence induction activity (D. Cahen *et al.*, in preparation).

The participation of polypeptides of both cytoplasmic and chloroplastic origins in the formation of photoactive PSI and II particles provides further evidence for the close interdependence and cooperation of both the cytoplasm and the plastid in the development of the photosynthetic apparatus.

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