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Short Communication

Polypeptide Composition of Photosynthetic Membranes from *Chlamydomonas reinhardi* and *Anabaena variabilis*

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ABSTRACT

***Anabaena variabilis*, a blue-green alga lacking chlorophyll *b*, shows an absence of the major 22 and 24 kilodalton polypeptides which are present in the photosynthetic membranes of *Chlamydomonas reinhardi* and higher plants. These data are consistent with other investigations which have shown that these polypeptides are associated with chlorophyll *b* in the chloroplasts of higher plants, and indicate the presence of a light harvesting chlorophyll-protein complex in higher plants which contains the chlorophyll *b* of the photosynthetic membrane.**

Recent experiments on the polypeptide composition of several subchloroplast fragments have shown differing general patterns for photosystem 1 and photosystem 2 fragments derived by the action of various detergents and French press treatment (1). Particles containing photosystem 1 contain several polypetide components, as revealed by SDS[1] gel electrophoresis, with major bands at about 62 and 52 kdaltons. Several bands are shown for photosystem 2 fragments, but all preparations show two major polypeptides at 22 and 24 kdaltons. In some runs these are not resolved and appear as one band at 23 kdalton. These two bands are clearly shown in the data obtained by Levine *et al.* (2) and are less clearly separated in our earlier experiments (1). It is now apparent that in the case of higher plants, these two polypeptides are associated with Chl *b*, and thus are associated with the light harvesting Chl-protein complex. This conclusion derives from the fact that these polypeptides are lacking from a barley mutant which lacks Chl *b*, and these represent the major difference between the profile of polypeptides from the chloroplasts of the wild type and mutant barley (6). Also, as shown below, these polypeptides are also lacking in preparations of TSF 2a from spinach, the reaction center complex from photosystem 2 which is depleted in Chl *b* by comparison to the initial photosystem 2 fragment.

Since these two polypeptides are associated with Chl *b* in chloroplasts of higher plants, it is of interest to investigate the blue-green algae for their presence. This communication reports that these polypeptides are lacking in membrane fragments of *Anabaena variabilis*, a blue-green alga. Earlier data presented by Levine *et al.* (2) and confirmed in this study, show that these two polypeptides are present in chloroplasts of *Chlamydomonas reinhardi*.

[1] Abbreviations: SDS: sodium dodecyl sulfate; TSF: Triton subchloroplast fragment.

MATERIALS AND METHODS

Anabaena variabilis cells were grown in modified Detmer's medium (4) in batches of 1.5 liters under fluorescent light of 5×10^3 ergs cm^{-2} sec^{-1} and a gas atmosphere of about 10% CO_2 in air. *Chlamydomonas reinhardi* cells were cultured in a medium described by Ohad *et al.* (5) under fluorescent light of 5×10^3 ergs cm^{-2} sec^{-1}. Photosynthetic membrane fragments of *A. variabilis* and *C. reinhardi* were prepared as reported previously (3), employing sonication of the algae prior to Triton X-100 treatment and separation on a sucrose gradient.

Chloroplasts and subchloroplast fragments of spinach obtained by treatment with Triton X-100 (TSF 1 and TSF 2) were prepared as described previously (7). Further treatment of the TSF 2 fragment, which contained photosystem 2, produced the TSF 2a particle which contains the reaction center complex of photosystem 2 (8).

SDS-acrylamide electrophoresis was performed essentially as described by Weber and Osborn (9). The membrane preparations were extracted with 80% acetone at −10 C prior to incubation with 1% SDS-10 mM sodium phosphate buffer, pH 7.0-1% mercaptoethanol. The ratio of SDS-protein was 5. The protein was solubilized upon incubation at 50 C for 2 hr or at room temperature overnight. The solubilized preparations were applied to the gel without removal of the insoluble residue, but almost all material entered the gel. Other details concerning the dimensions of the gel and electrophoretic operations were as previously reported (1). Densitometer tracings were obtained with a Schoeffel spectrodensitometer Model SD 3000.

RESULTS AND DISCUSSIONS

Figure 1 shows the polypeptide distribution obtained for washed chloroplast lamellae. Since these membranes were not treated with dilute salt or EDTA, coupling factor was retained on the membrane, as evidenced by the major band at 58 kdalton. The band at 62 kdalton, characteristic of photosystem 1, is present as a shoulder on the 58 kdalton band, and the two bands at 22 and 24 kdaltons, characteristic of photosystem 2, are evident.

Figure 2 shows the polypeptide composition of the TSF 2 fragment obtained with Triton X-100 which has been treated to extract the TSF 2a particle. The TSF 2 fragment contains photosystem 2 activity and is enriched in Chl *b*, with a Chl *a*/Chl *b* ratio of 1.8. Also shown in Figure 2 is the polypeptide composition of the TSF 2a particle, which contains the reaction center complex of photosystem 2, but is depleted in Chl *b* with a Chl *a*/Chl *b* ratio of 8. It is apparent that the major difference between the TSF 2 and TSF 2a polypeptide patterns is the absence of the 22 and 24 kdalton polypeptides in the TSF 2a particle which lacks Chl *b*. This agrees with the data reported by Thornber and Highkin (6) for a barley mutant

lacking Chl *b*. The barley mutant also lacks the 22 and 24 kdalton polypeptides.

Figure 3 shows the polypeptide composition of the green alga *Chlamydomonas reinhardi*, and also shows similar data for the photosynthetic membrane prepared from *Anabaena variabilis*. Comparison of the polypeptide patterns of spinach and *Chlamydomonas* preparations shows a similar pattern in both cases, with the 22 and 24 kdalton polypeptides occurring as major bands. This confirms the earlier data of Levine *et al.* (2), who by using the Hoober method of electrophoresis obtained sharper separations of the various polypeptides.

Comparison of the two polypeptide profiles of Figure 3 shows that in *Anabaena variabilis*, the 22 and 24 kdalton polypeptide components are missing. The other bands agree quite well with those found with *Chlamydomonas*. Since *Anabaena*, along with other blue-green algae, lacks Chl *b*, these data are consistent with what has been learned about the structure of the photosynthetic membrane in higher plants. Chl *b* is lacking in the blue-greens, and since the 22 and 24 kdalton polypeptide bands are also missing, it is not possible to do experiments similar to those done with mutants or with subchloroplast fragments of higher plants. With higher plants experiments have shown the presence of Chl *b* is associated with the 22 and 24 kdalton polypeptide components, leading to the presumption that these polypeptides do contain Chl *b* in the form of a light harvesting Chl protein. Isolation of this light harvesting Chl protein will allow a definite answer to this problem. At present, however, the data obtained with a representative blue-green alga are consistent with the hypothesis that the 22 and 24 kdalton polypeptides are associated with Chl *a* and Chl *b* in a light-harvesting Chl protein complex which is structurally associated with photosystem 2.

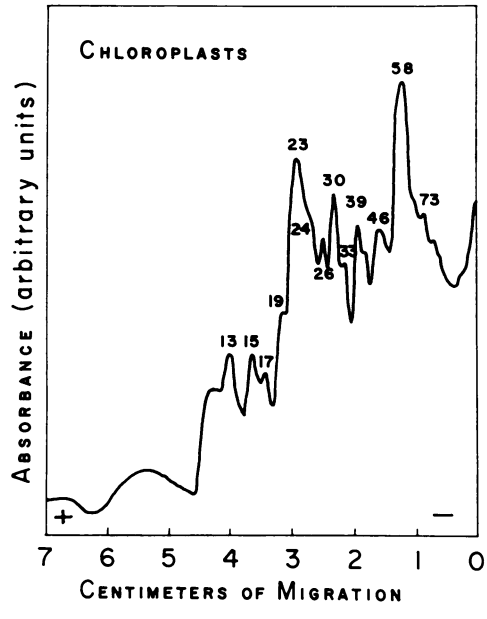

FIG. 1. Polypeptide distribution pattern of spinach chloroplast lamellae obtained by SDS-polyacrylamide gel electrophoresis. Numbers indicate mol wt. $\times 10^{-3}$.

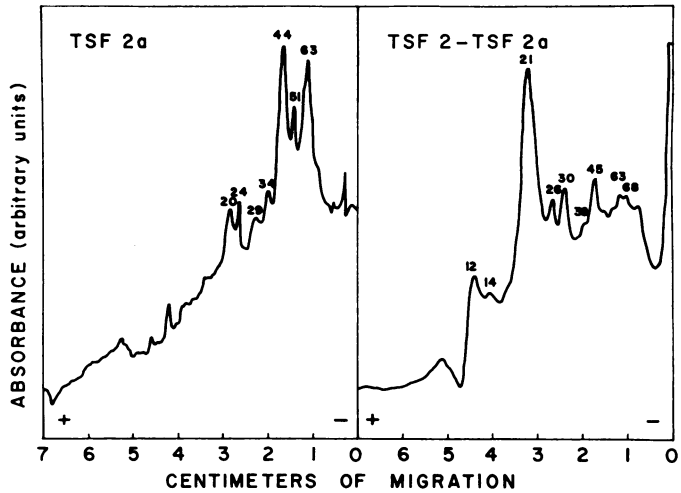

FIG. 2. Polypeptide distribution pattern obtained by SDS-polyacrylamide gel electrophoresis of TSF 2a fragment and the residual TSF 2 after removal of the TSF 2a particle.

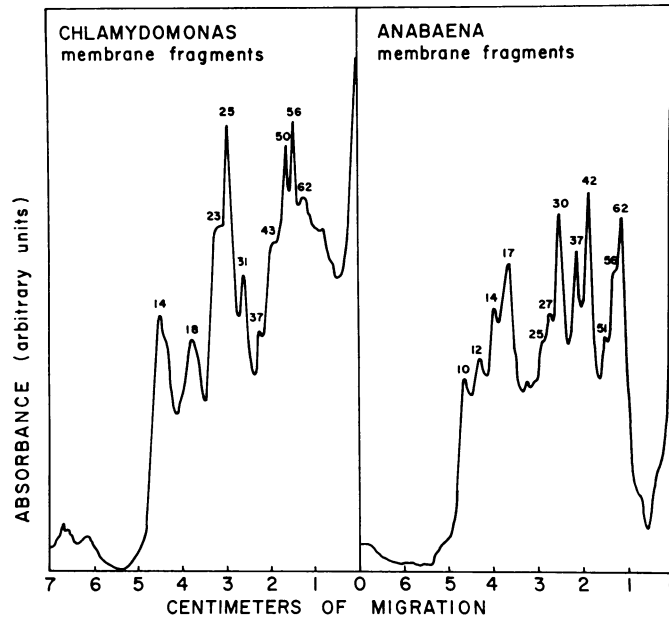

FIG. 3. Polypeptide distribution pattern obtained by SDS-polyacrylamide gel electrophoresis of photosynthetic membrane fragments from *C. reinhardi* and *A. variabilis*.

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