Fractionation and Analysis of Polypeptides of *Euglena gracilis* Chloroplasts

**AUREA C. VASCONCELOS, LETICIA R. MENDIOLA-MORGENTHALER, GARY L. FLOYD,** and **JEFFREY L. SALISBURY**

*Department of Botany and Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, New Jersey 08903*

**ABSTRACT**

Intact *Euglena gracilis* chloroplasts, purified on gradients of silica sol, were lysed osmotically and fractionated by centrifugation on discontinuous gradients of sucrose into their soluble, envelope membrane, and thylakoid membrane components. The proteins of the different subchloroplast fractions, as well as those of whole chloroplasts, were analyzed by electrophoresis on sodium dodecyl sulfate polyacrylamide gels. The polypeptide profile of each fraction was distinctive and was in general similar to the profile obtained for analogous fractions of the chloroplasts of higher plants.

The envelope membranes were separated into two fractions in the gradients according to their banding densities. Electron micrographs showed that the light envelope fraction consisted mostly of single-membrane vesicles, whereas the heavy envelope fraction consisted of multiple layers of folded membranes. Both envelope fractions were ultrastructurally distinct from the thylakoid membranes.

Chloroplast development in *Euglena* provides a useful experimental framework within which to study interactions between two distinct informational systems of eukaryotic cells. The chloroplasts contain their own DNA and a protein synthesizing machinery distinct from that of the cytoplasm where the products of nuclear genes are translated.

In order to study informational interactions occurring during chloroplast replication or development, such as those in the transition from dark to light, it is necessary to establish a system which allows a repeatable pattern of chloroplast isolation, fractionation, and the characterization of the polypeptides of the organelle.

We recently described a method for the isolation of intact chloroplasts of *Euglena* in high yield and purity (18). Other methods have been developed recently for the subfractionation of chloroplasts into soluble, thylakoid, and envelope components (4, 16), and the subsequent analysis of the polypeptides in these fractions by SDS-polyacrylamide gel electrophoresis (13, 15). In this paper we employ these procedures for the fractionation and analysis of *Euglena* chloroplasts. The ultrastructure of the membrane fractions is also examined by electron microscopy.

**MATERIALS AND METHODS**

**Preparation of Chloroplast Fractions.** Chloroplasts were prepared as described previously (18) from photothermotrophically grown *Euglena gracilis* (Klebs) strain Z (Pringsheim) by isopycnic sedimentation in gradients of Ludox AM, a commercially available silica sol. Intact chloroplasts from the lower band in the Ludox gradients were pooled and washed twice. The pelleted chloroplasts were lysed osmotically by resuspending them in Tricine/KCl buffer (25 mM Tricine:12.5 mM KCl, pH 6.8) stirred with a magnetic bar for 20 min at 4 C, and homogenized with 10 strokes of a TenBroeck homogenizer. The lysed chloroplasts were fractionated on discontinuous gradients of sucrose (12:23:30%, w/v) as described by Poncelet and Day (16) for the fractionation of spinach chloroplast envelopes, except that the sucrose was buffered with 25 mM Tricine:12.5 mM KCl, pH 7.6. Centrifugation was carried out at 27,000 rpm for 1 hr in a Damon/IEC SB-283 rotor. The two bands of envelope membranes at the interfaces between 12 and 23% sucrose and between 23 and 30% sucrose were collected, diluted with Tricine/KCl buffer, and washed by centrifugation at 48,000 rpm in a Damon/IEC A-321 rotor for 40 min.

A nonsedimenting zone containing soluble proteins was collected by aspiration. A green pellet at the bottom of the tube contained thylakoid membranes and was resuspended and washed twice with Tricine/KCl buffer. Half of the thylakoid fraction was extracted with 80% acetone to extract the pigments present in these membranes (7).

**Analysis of Polypeptides by Electrophoresis on SDS-Polyacrylamide Gels.** Polyacrylamide gels containing 0.1% SDS (w/v) were prepared essentially according to the method of Laemmli (10). The separation gel contained 12.5% acrylamide and 0.33% (w/v) N,N-methylene-bis-acrylamide, whereas the stacking gel contained 3% (w/v) acrylamide. The gels were set in glass tubes 17 cm long with an internal diameter of 0.5 cm.

Samples for electrophoresis were taken up in sample buffer consisting of 0.0625 M tris-HCl, pH 6.8, 5% (v/v) β-mercaptoethanol, 3% (w/v) SDS, and 10% (v/v) glycerol and heated at 70 to 80 C for 10 min. The conditions for electrophoresis were described before for the polypeptides of spinach chloroplasts (13).

The gels were fixed and stained with Coomassie Brilliant Blue following the method outlined by Fairbanks et al. (6). The mol wt of the polypeptides were estimated from the mobilities of standard proteins, according to Weber and Osborn (22).

**Preparation of Fractions for Electron Microscopy.** Envelopes were collected from the sucrose gradients and diluted with Tricine/KCl buffer containing 4% glutaraldehyde to give a final concentration of 2% of glutaraldehyde. They were washed twice and fixed at the same time by centrifugation at 48,000 rpm in a Damon/IEC A-321 rotor for 40 min. The thylakoid fractions

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2 Present address: Department of Botany, School of Biological Sciences, Ohio State University, Columbus, Ohio 43210.
RESULTS AND DISCUSSION

Chloroplasts from *Euglena gracilis* were purified by sedimentation in gradients of colloidal silica, lysed osmotically, and centrifuged on discontinuous gradients of sucrose as described under “Materials and Methods.” Soluble proteins remained above the sucrose gradient, the thylakoid membranes penetrated to the bottom, where they formed a green pellet, and the envelope membranes banded at two intermediate densities, one band at the interface between 12 and 23% sucrose and the other at the interface between 23 and 30% sucrose. These were designated as the light and heavy fractions of the envelope membranes, respectively. Both membrane fractions yielded yellow pellets that were visibly free of Chl after recovery from the gradients and recentrifugation.

Electron micrographs of the different membrane fractions showed that the thylakoid membranes were ultrastructurally distinct from the membranes of the envelope, and that the latter membranes also differed in their conformation, depending on their banding densities (Fig. 1). The light envelope fraction appeared to consist mostly of single membrane vesicles (Fig. 1A), whereas the heavy envelope fraction contained multiple layers of folded membranes (Fig. 1B). Poncelet and Day (16) had previously reported that the light and heavy envelope fractions of spinach chloroplasts consisted primarily of single and double membrane vesicles, respectively. It should be noted that varying types of structures have been reported for the envelope membranes which had been isolated by different procedures for the chloroplasts of spinach and other higher plants (4, 8, 12, 20).

Our electron micrograph of the heavy envelope fraction in Figure 1B closely resembles the packed, folded membranes of spinach chloroplast envelopes reported by Douce *et al.* (4).

The thylakoid fraction (Fig. 1C) showed a more regular array of lamellae with densely staining particles, which could be ribosomes still attached to the membranes. Schwartzbach *et al.* (19) had previously observed that mechanical breakage of *Euglena* chloroplasts failed to release significant amounts of ribosomal material from the membrane fractions.

The polypeptide profiles obtained after electrophoresis on SDS-polyacrylamide gels were also distinctive for each subchloroplast fraction. As might be expected, whole, unfractionated chloroplasts showed a complex set of polypeptides ranging from about 12 to 100 kilodaltons in mol wt (Fig. 2A). The more prominent polypeptides are found in the regions of the gel corresponding to 60 to 48 kilodaltons and around 28 to 22 kilodaltons in mol wt.

The soluble proteins which remained in the sample zone at the top of the gradients also contained numerous polypeptides (Fig. 2B). Characteristically, the major components were at about 52 and 12 kilodaltons, corresponding to the large and small subunits, respectively, of ribulose diphosphate carboxylase. The electrophoretic behavior of ribulose diphosphate carboxylase was previously determined with samples of partially purified preparations of the enzyme from *Euglena* (unpublished). This enzyme protein was also found to predominate in the polypeptide profiles of the soluble proteins of higher plant chloroplasts (2, 3, 13-15).

The polypeptide profiles obtained for the thylakoid membranes before and after extraction with acetone are shown in

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**Figure 1.** Electron micrographs of *Euglena* chloroplast membrane fractions obtained from sucrose gradients. The calibration bar represents 0.5 μm. **A:** light envelope membranes; **B:** heavy envelope membranes; **C:** thylakoid membranes.

**Figure 3.** The most prominent polypeptides are found in the regions of the gel corresponding to about 60 to 45 kilodaltons and another set at about 30 to 22 kilodaltons. These groups of polypeptides may be analogous to the groups I and II polypep-
tides believed to be associated with the protein-Chl complexes of photosystems I and II, respectively, of other photosynthetic organisms (1, 9, 11, 17). Prior extraction of the thylakoid membranes with acetone resulted in the loss or decrease of certain polypeptides (shown by arrows in Fig. 3B).

The set of envelope membrane proteins (Fig. 4) is again quite distinct from that of the thylakoid membranes or of the soluble fraction of the chloroplast. In contrast to the other compartments of the chloroplast, most of the polypeptides of the envelope membranes are found at upper regions of the gel corresponding to mol wt about 100 to 26 kilodaltons. Similar observations were reported for the polypeptides of the chloroplast envelope membranes of spinach (13) and of pea (8). There are in addition two rather broad bands, one at about 10 kilodaltons and the other just before the marker dye. The identity of the former is not known, although Pineau and Douce (15) had reported that their spinach chloroplast envelope membrane preparations contained a discrete polypeptide whose electrophoretic mobility was identical to that of the small subunit of ribulose diphosphate carboxylase. Unlike the results obtained with spinach (13), the polypeptide profiles for the light envelope fractions were identical to those of the heavy envelope fractions (Fig. 4, A and B).

Considering the ultrastructural differences between the two envelope membrane fractions of Euglena chloroplasts, the identical polypeptide profiles could be interpreted to mean that the single membrane vesicles of the light envelope fraction consisted of both outer and inner envelope membranes which were separated from each other during fractionation. Alternately, if the single membrane vesicles of the light envelope fraction represent mostly outer envelope membranes as had been suggested in the case of spinach chloroplast (13, 20), our results could also mean that the polypeptide composition of the outer and inner envelope membranes of Euglena are identical.

The polypeptide profiles of the soluble, thylakoid, and envelope membrane components of chloroplasts of Euglena, spinach (13, 15), and pea (2, 5, 8) have the following features in common. (a) The predominant polypeptides among the soluble proteins are the large and small subunits of ribulose diphosphate carboxylase. (b) The thylakoid membranes contain two major polypeptide size classes, analogous to the group I polypeptides and group II polypeptides of higher plants and Chlamydomonas (1, 11). (c) The envelope membrane polypeptides are mostly of higher mol wt.

When we compare the polypeptides of the envelope membranes of Euglena chloroplast with our earlier analyses of spinach chloroplast envelopes which were performed under nearly identical conditions, we see the profiles to be qualitatively very similar with only some quantitative differences.

In summary, we find that to the extent that comparisons are possible, the proteins of the chloroplasts of Euglena, a protist of uncertain phylogenic affinities (21), are similar to those of the chloroplasts of higher plants and of the green alga Chlamydomonas. The similarity is expressed in terms of the polypeptide size class composition among the different subchloroplast frac-

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**Fig. 3.** SDS-polyacrylamide gel electrophoretic patterns of thylakoid membrane polypeptides. A: thylakoid membranes before extraction with acetone; B: thylakoid membranes previously extracted with acetone; the arrows indicate the polypeptides which show quantitative losses after solvent extraction.

**Fig. 2.** SDS-polyacrylamide gel electrophoretic patterns of the polypeptides of Euglena chloroplasts. Gel patterns of several preparations yielded reproducible results in all fractions analyzed. A: whole, unfractionated chloroplasts; B: soluble fraction.
Fig. 4. SDS-polyacrylamide gel electrophoretic patterns of envelope polypeptides. A: light envelope fraction; B: heavy envelope fraction.

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LITERATURE CITED


