Structural Characteristics of a Photosynthetic Mutant of Euglena gracilis Blocked in Photosystem II

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ABSTRACT

The techniques of thin sectioning and freeze etching were employed in comparing the chloroplast structure of the wild type and photosynthetic mutant P. of Euglena gracilis, Z strain. The mutant chloroplasts were characterized by a lack of thylakoid pairing even under high salt conditions. In addition the mutant thylakoids were more varied in size and fewer in number than those of the wild type. No differences between the mutant and wild type were observed in the size and distribution of the particles within the chloroplast membranes seen by the freeze-etching technique.

These structural abnormalities do not appear to be correlated per se with the absence of plastoquinone A in the mutant but may be related to a different lipid composition observed in the mutant.

The mutant P, of Euglena gracilis, Z strain, was first isolated and studied by Russell and Lyman (15) and Russell et al. (16). They suggested that the mutation blocked in the electron transport chain between the two photosystems and that the mutant had a missing or nonfunctional primary electron acceptor for photosystem II.

In an accompanying paper (18) we report confirmation of their results by other biochemical and biophysical techniques. Furthermore, we showed that the mutant P, lacked plastoquinone A but possessed plastoquinone B in contrast to the wild type, which like other green algae studied (21), has plastoquinone A but not plastoquinone B. Cytochrome b₅₆₃ seemed to be absent or nonfunctional in the mutant. The mutant had a higher complement of carotenoids, especially xanthophylls, and probably a different composition of other lipids from that of the wild type.

This structural study was undertaken to ascertain if the morphology of the thylakoid membranes was altered by the mutation. An attempt was made to correlate biochemical data with the structural studies.

Materials and Methods

Culture and Maintenance of Algal Strains. These methods are described in an accompanying paper (18).

Isolation of Algal Chloroplasts. Refer to accompanying paper (18).

Electron Microscopy. The algal samples were fixed in a solution containing 2% (w/v) paraformaldehyde, 2% (v/v) glutaraldehyde, 75 mM cacodylate buffer pH 7.4, and 50 mM sucrose (for whole cells) or 0.3 M sucrose (for isolated chloroplasts) for 2 hr at 0 C. Postfixation was carried out in either 1% (v/v) osmium tetroxide for 2 hr or 1% (w/v) aqueous potassium permanganate for 1 hr in the cold. The samples then were usually stained overnight in uranyl acetate. After being dehydrated in acetone, the samples were embedded in an Epon-Araldite epoxy resin mixture (9). Silver-grey sections were cut with a diamond knife and poststained with lead citrate.

The samples were prepared for freeze etching as described previously (19). A small drop of the sample was frozen in a gold cup and fractured in a freezing microtome according to the procedure reported by Moor and Mühlethaler (11).

All samples were examined with a Philips EM 200 electron microscope.

High and Low Salt Treatment of Chloroplasts. The algal chloroplasts were isolated as described above. The high salt treatment consisted of washing the chloroplasts over a period of 70 min in a solution containing 0.1 M KCl, 0.4 M sucrose, and 50 mM Tricine, pH 7.4. The low salt treatment was carried out in the same manner but in a solution containing 0.4 M sucrose and 20 mM Tricine, pH 7.4. After the samples had undergone the salt treatment, they were fixed for electron microscopy in a solution that contained: 2% (v/v) glutaraldehyde, 2% (v/v) paraformaldehyde, the appropriate high or low salt content, and 0.4 M sucrose. The rest of the fixation and embedding procedure was as described above.

RESULTS

Thin sections of wild-type and mutant P, cells grown in the dark showed no differences in general structure or in the plastids (17). However, after approximately 72 hr in the light, when chloroplast development is near completion, the appearance of the wild-type chloroplast by thin section (Fig. 1) is definitely different from that of the mutant P, chloroplasts (Fig. 2). The chloroplasts of the wild-type strain are characterized by thylakoid pairing (Fig. 1), whereas the thylakoids of the mutant strain are essentially unpaired (Fig. 2). This same pattern of paired thylakoids in the wild type and unpaired thylakoids in the mutant was confirmed in

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Fig. 1. Thin section of mature wild-type chloroplast. The thylakoids (arrow) are characteristically paired. × 20,625.

Fig. 2. Thin section of mature P₁ chloroplast. The thylakoids (arrow) appear unpaired and sometimes discontinuous. × 35,779.

Fig. 3. Freeze-etched wild-type chloroplast. The paired thylakoids (arrow) appear as four lines closely parallel to one another. × 69,000.

Fig. 4. Freeze-etched P₁ chloroplast. In this view of the mutant chloroplast the unpaired nature of the thylakoids (arrow) can be observed. × 69,000.
freeze-etched fractures of wild-type (Fig. 3) and P₄ (Fig. 4) chloroplasts. In these freeze-etched surfaces the common occurrence of two closely appressed thylakoids in the wild type (Fig. 3) is in contrast to single thylakoids (Fig. 4) in the mutant.

Both thin sections (Fig. 1, 2) and freeze-etched replicas (Fig. 3, 4) reveal fewer thylakoids in the mutant as compared to the wild type. The lengths of the individual thylakoids in the mutant vary considerably more than in the wild type.

No differences were detected in the size and distribution of the particles associated with the thylakoid and observed by the freeze-etching technique. The wild-type chloroplast (Fig. 5) shows characteristic two arrangements of particles, closely packed and widely dispersed (4). The same sized particles (approximately 110 Å) and similar distribution of these particles are seen associated with the thylakoids of the mutant Figure 6.

The response of the Euglena chloroplasts of the wild-type strain to high and low salt conditions corresponded to that seen in higher plants by Izawa and Good (7). The wild-type thylakoids unpaired when in a low salt solution (Fig. 7) while they re-paired upon subsequent washing in a high salt solution (Fig. 8). However, the thylakoids of the mutant remained unstacked when washed in high salt (Fig. 9).

**DISCUSSION**

Homan and Schmid (23) reported that mutants of Nicotiana tabacum which lacked the oxygen-evolving apparatus of photosystem II had unstacked thylakoid membranes. They concluded that a close packing of at least two thylakoids was required for photosystem II activity and suggested that stacking creates a hydrophobic environment where the oxidation of water can occur. However, Goodenough and Levine (2) described a pale green mutant strain of Chlamydomonas reinhardi, ac-31, characterized by the absence of any stacking of its chloroplast membranes, but with a substantial capacity for photosynthetic electron transport, photophosphorylation, and carbon fixation. Their experiments suggest rather that membrane stacking is required for enhanced energy transfer. Goodenough and Staehelin (3) further showed that when the chloroplasts of this mutant strain are isolated in high salt medium (7), membrane stacking occurs in the chloroplasts. They concluded that the ability of membranes to stack can be modified by the ionic environment in which the chloroplast membranes are found. However, the lack of thylakoid stacking in the mutant P₄ of Euglena gracilis seems to be more complex. The wild-type chloroplasts reacted as did those of higher plants under high and low salt treatments (7). The wild-type thylakoids unpaired in low salt solution and subsequently re-paired in a high salt solution. The fact that the mutant chloroplasts remained unpaired in a high salt solution indicates that a lack of a proper ionic environment is not responsible for the absence of thylakoid pairing in the mutant P₄ strain of Euglena.

The significance of the particles within the chloroplast membrane, seen by the freeze-etching technique, is still not clear. Arnzen et al. (1) related these structures in spinach to the light-induced reactions of photosynthesis and showed that the surface with large particles (approximately 180 Å) has...
Fig. 7. Thin section of wild-type chloroplast under low salt conditions. The usually paired thylakoids (arrow) of the wild-type chloroplast become unpaired under low salt-conditions. × 24,975.

Fig. 8. Thin section of wild-type chloroplast under high salt conditions. The wild-type thylakoids (arrow) re-pair when washed in high salt after a low salt treatment. × 28,333.

Fig. 9. Thin section of P₄ chloroplast under high salt conditions. The thylakoids (arrow) remain generally unpaired under high salt conditions. × 40,800.
photosystem II activity while photosystem I activity is associated with the membrane surface containing the small particles (approximately 110 Å). On the other hand Goodenough and Staehelin (3) have evidence that in *Chlamydomonas* the large particle represents a stacking factor. Our observations (17), as well as others (4), have shown particles associated with the chloroplast membranes of *Euglena*. Our data indicate that both distributions of particles in *Euglena*—the closely packed and widely dispersed—contain the same sized particle, of approximately 110 Å (17). Phung nhu Hung *et al.* (12) found a particle size and distribution, similar to that in the mature *Euglena* chloroplasts, in developing barley chloroplasts during the early stages of greening. Only later in chloroplast development did the large and small particles, characteristic of higher plants, appear: the large particles were found only in the stacked regions. A similar observation in bean was made by Remy (13). There is no large particle in *Euglena*, such as that seen in higher plants by freeze-etching, although chloroplast membranes do pair in the wild type cells. Furthermore, the mutant chloroplasts, which do not have thylakoïd pairing, have a particle size and distribution similar to that of the wild-type chloroplasts. Hence it does not appear that a particular type of particle seen in freeze-etched surfaces of chloroplasts can be identified as a stacking factor in *Euglena*.

Since the mutant has been shown to lack plastocyanin a, it is tempting to try to correlate structural changes with the lack of plastocyanine A. However, it is probable that plastocyanine A is not involved directly in membrane pairing since Barr, Hall, and Crane (personal communication) described a *Chlorella* mutant that was deficient in plastocyanine A and possessed membrane pairing in the chloroplasts. On the other hand, Keck *et al.* (26) reported on a soybean mutant which had a high plastocyanine content but had less pairing of membranes than did the wild type. However, this particular mutant had less lipids of certain types (monogalactosyl and digalactosyl diglycerides) than did the wild type. Since there is a change in lipid composition during chloroplast development (14) and lipids are an integral part of the thylakoids, lipids may be involved in this unpairing phenomenon as well as other organizational aspects of the chloroplast structure. Both the chromatography and the spectra revealed that the wild-type cells differed from the mutant cells in the content of other lipids as well as quinones and carotenoids. These data were only visual and comparative; more studies will have to be undertaken for more specific information on the kinds and amounts of lipids present.

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LITERATURE CITED


