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Cooperation of Cytoplasmic and Plastidial Translation in Formation of the Photosynthetic Apparatus and Its Stage-Specific Efficiency[1]

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ABSTRACT

In synchronized *Euglena gracilis*, strain Z, the synthesis of the apoproteins for the chlorophyll-protein-complexes CPI, CPa, and LHCP is light-dependent and takes place in the light period in a characteristic consecutive fashion: CPI at 1 to 2 hours, CPa at 7 to 12 hours, and LHCP at 8 to 12 hours. The syntheses sequence of the chlorophyll-protein-complexes coincides with the efficiency alterations of the photosynthetic apparatus of *E. gracilis* during the light period of the cell cycle. In particular, the synthesis onset of the photosystem II-related polypeptides CPa and LHCP aligns with the decrease of oxygen evolution at 6 hours of the light period and is discussed as reorganization process in the thylakoids.

The biogenesis of *Euglena* chloroplasts is a complicated, highly organized, and well-regulated process (3–9, 15, 21) which is dependent on both chloroplast and cytoplasmic protein synthesis (5–8). It is clear that the formation of thylakoids in *Euglena* chloroplasts requires the integrated activities of both translation systems.

From regreening experiments of etiolated organisms, it is well documented (11, 13, 17, 20) that thylakoid formation is a stepwise assembly process by which first a membrane is built up containing a certain minimal number of components, with other constituents being added later when construction has reached the appropriate stage. The different stages in the development of fully functional thylakoid membranes can be distinguished during the first phase of greening only because at this stage all the plastids are in synchrony and will tend to go through the stages of thylakoid development together. The degree of synchrony no doubt is diminishing with time. Therefore, the synchronized cell development during the whole cell cycle is an essential prerequisite to study the stepwise thylakoid development in *Euglena* chloroplasts (5, 6) and to point out the cooperation of plastidial and cytoplasmic translation.

The aim of this work is to identify steps in thylakoid formation of the photosynthetic apparatus in synchronized *Euglena gracilis*, their dependence on the light regime, the interplay of plastidial and cytoplasmic translation of proteins for the Chl-protein complexes CPI[2] (5), CPa (22), and LHCP (5), and the resulting efficiency in O_2 evolution.

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[2] Abbreviations: CPI, Chl-protein-complex I related to PSI; CPa, PSII-associated Chl *a*-protein; LHCP, light-harvesting Chl *a/b* protein; LH, hour of the light period of the cell cycle.

MATERIALS AND METHODS

Organism and Growth. *Euglena gracilis*, strain Z (Sammlung von Algenkulturen, Pflanzenphysiologisches Institut, Universität Göttingen), was cultured phototrophically (24) and synchronized by daily light-dark periods of 14 h light and 10 h dark with dilution of the culture at the end of the dark period.

Measurement of Photosynthetic O_2 Evolution. Net O_2 evolution of whole *Euglena* cells was measured directly in the phototrophic medium with a Clark electrode at saturating light intensity (32.026 w m^{-2}) (10). Chl was measured according to Arnon (2).

Protein Synthesis and Electrophoresis. Light-dependent protein synthesis by *Euglena* cells was measured in the presence of ^{14}C-amino acid mixture (1.85 GBq/milliatom) (Amersham) at 27°C with gentle shaking under illumination with white light (32.026 w m^{-2}). Isolation of *Euglena* chloroplasts and separation of purified thylakoids were performed according to Brandt (5). The thylakoids were solubilized at 4°C in enough sample buffer (1 M Tris-HCl [pH 6.8] containing 10% glycerol and 1% SDS) to give a final SDS:Chl weight ratio of 10:1 (w/w). The solubilized thylakoids were applied immediately into the sample wells of the SDS slab gels. Gel preparation and electrophoretic run was performed at 4°C for 3 h at 30 mamp constant current (5). Gel slices containing CPI, CPa, or LHCP were solubilized in 1 ml NCS for 5 h. Then, 5 ml Aquasol was added and activity was counted in a Liquid Scintillation Spectrometer, model 2450 Tri-Carb (Packard Instruments Company).

In control experiments, the Chl-protein complexes CPI, CPa, and LHCP were isolated by SDS-gel electrophoresis as well as by isoelectrofocusing and SDS-reelectrophoresis. Both methods demonstrate the absence of other polypeptides in the region of the Chl-protein complexes. During the cell cycle, 8% of the ^{14}C incorporation was found in the pigments of the Chl-protein complexes determined by ^{14}C measurement of 'green bands' and of 'protein-bands' after pigment extraction.

RESULTS

The chloroplast biogenesis in synchronized *E. gracilis* takes place during the light-time of the cell cycle, but neither the chloroplasts nor the *Euglena* cells divide (Fig. 1; Ref. 21). Total protein of *Euglena* cells doubles in a linear fashion during this light period (10). Synthesis of special proteins, however, like the apoproteins of the Chl-protein-complexes CPI, CPa, and LHCP occurs at distinct stages of the cell cycle only (Fig. 2).

(a) Synthesis of the Chl-protein-complex of PSI, CPI with an apparent M_r of 110,000 (Fig. 3) (5) and an absorption maximum at 674 nm (Fig. 4; Ref. 22), occurs at 1 to 2 h of the light-time (LH) (Fig. 2a) and is light-dependent (Fig. 2b). An additional dark period of 3 h at the beginning of the light period causes a

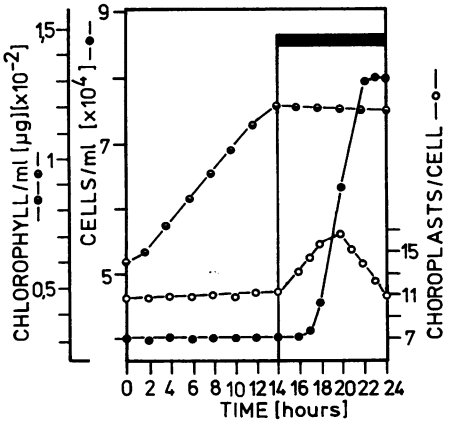

FIG. 1. Cells/ml (●) and chloroplasts/cell (○) of a *E. gracilis* culture synchronized by light-dark change of 14 h light and 10 h dark.

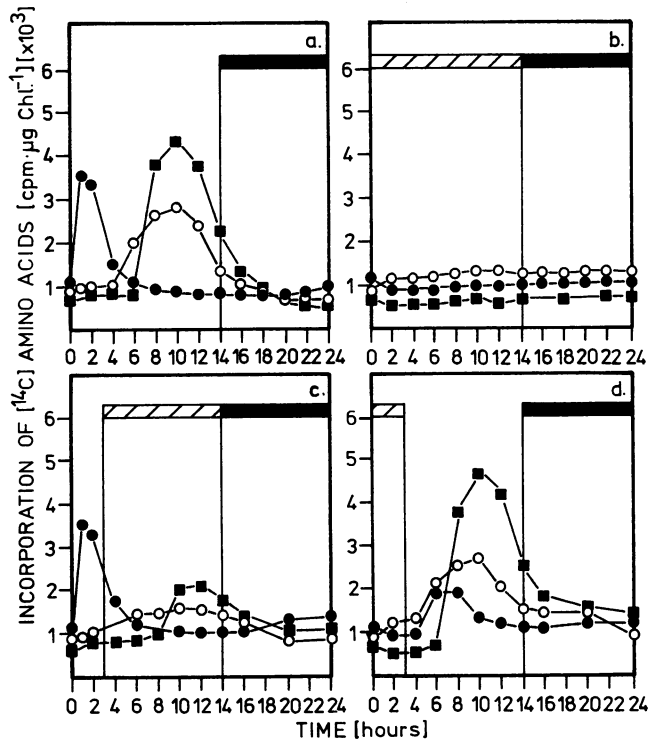

FIG. 2. Incorporation of ^{14}C-amino acids in the thylakoid Chl-protein-complexes CPI (●), CPa (○), and LHCP (■) of synchronized *E. gracilis*, strain Z, during the cell cycle with 14 h light and 10 h dark (a), during the cell cycle with additional dark during the light period (b), with additional dark at 3 to 14 h of the light period (LH), (c) or with additional dark at 0 to 3 LH (d).

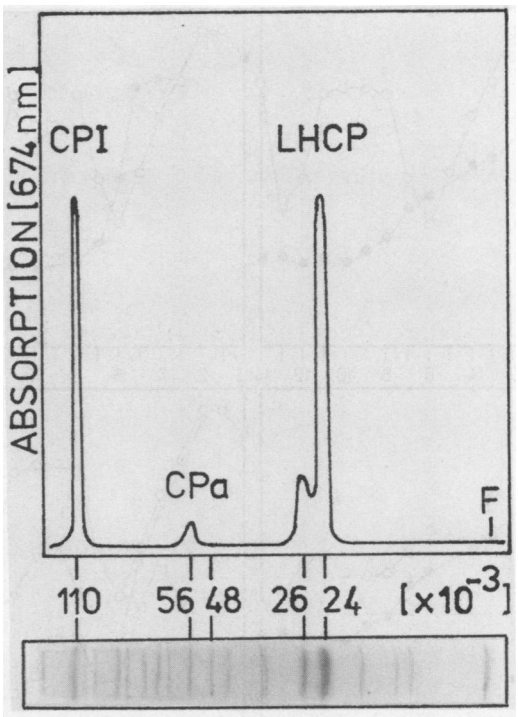

FIG. 3. Absorption of the Chl-protein-complexes CPI, CPa, and LHCP of *E. gracilis*, strain Z, separated by SDS-polyacrylamide gel electrophoresis and protein-spectrum after Coomassie blue staining.

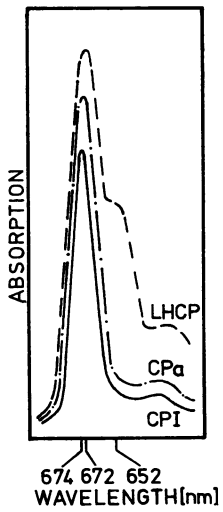

FIG. 4. Absorption spectra of CPI, CPa, and LHCP of *E. gracilis*, strain Z, isolated by SDS-polyacrylamide gel electrophoresis.

shift of the CPI synthesis to 4 to 7 LH and a decrease to about 50% (Fig. 2d).

(b) Synthesis of the Chl-protein-complex of PSII, CPa with two apoproteins (57 and 48 kD) (Fig. 3; Ref. 22) and an absorption maximum at 674 nm (Fig. 4; Ref. 22) occurs at 7 to 12 LH (Fig. 2a) and is light-dependent (Fig. 2b). An additional dark period of 3 h at the beginning of the light period causes no significant alterations (Fig. 2d); whereas, in an additional dark period of 11 h at 3 to 14 LH, the synthesis of CPa-apoproteins decreases to about 10% (Fig. 2c).

(c) Assembly of the two light-harvesting Chl *a/b* proteins of 26 and 24 kD (Fig. 3; Ref. 5) with absorption maxima at 672 and 652 nm (Fig. 4; Ref. 22) occurs at 8 to 12 LH (Fig. 2a) and is light-dependent too (Fig. 2b). Its behavior in additional dark periods (Fig. 2, c and d) is like the synthesis of CPa. Before attempting any study of the photosynthetic efficiency of *Euglena* chloroplasts *in vivo* (measured as photosynthetic O_2-evolution) with respect to this succession of syntheses, it was important to investigate the behavior of the 'water-splitting enzyme' in *E. gracilis* chloroplasts. The process of photosynthetic water oxidation can be schematically described by the Kok-scheme (14), but little is known about the chemical nature. Especially in *E. gracilis*, the repair of H_2O splitting activity is accompanied by synthesis of some polypeptides in the mol wt range of 50,000 to 60,000 (11). The thylakoid content of this group of polypeptides as well as of two other polypeptides (M_r of 23,000 and 16,000) presumably related to the water-splitting enzyme (1) increases very slightly during the light period of the cell cycle (data not shown) and cannot effect significant alterations

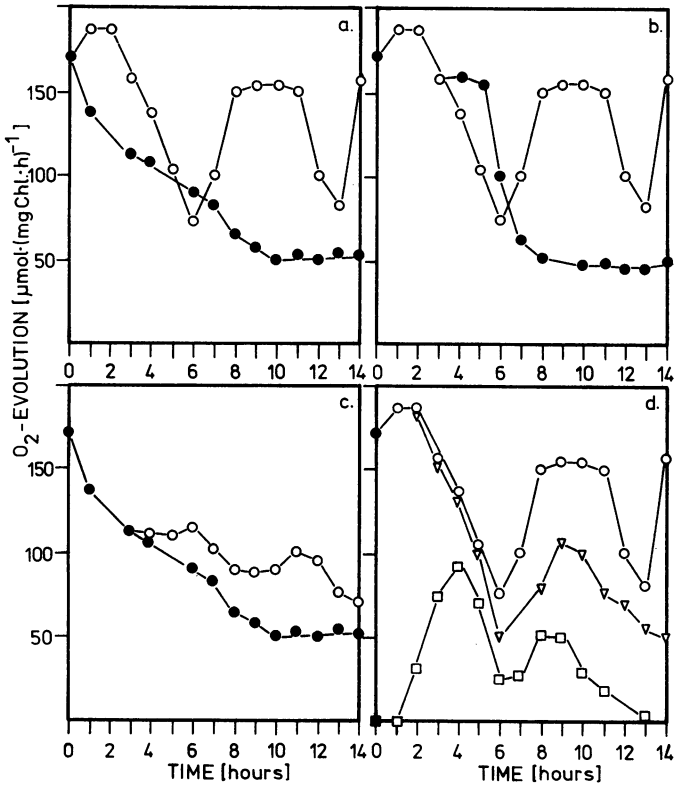

FIG. 5. Photosynthetic O_2 evolution (○) of synchronized *E. gracilis*, strain Z, during the light period (14 h) of the cell cycle (24 h). Light intensity 32.026 w m^{-2}. a, (●), Additional dark period at 0 to 14 h of the light period (LH). b, (●), Additional dark period at 3 to 14 h LH. c, (●), Additional dark period at 0 to 3 h LH. d, O_2 evolution after treatment with cycloheximide (▽) or streptomycin (□) for 1 h.

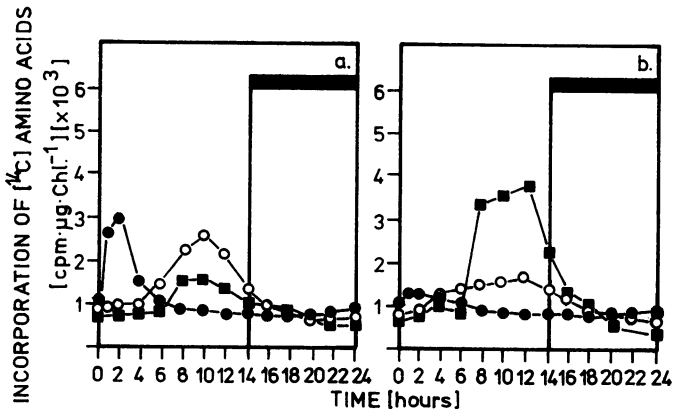

FIG. 6. Incorporation of ^{14}C-amino acids in the thylakoid Chl-protein-complexes CPI (●), CPa (○), and LHCP (■) of synchronized *E. gracilis*, strain Z, after incubation with cycloheximide (a) or streptomycin (b) for 1 h.

in photosynthetic O_2 evolution.

Photosynthetic O_2 evolution by *E. gracilis*, however, changes remarkably during the light period (Fig. 5a) and is no reflection of Chl accumulation (Fig. 1). In particular, O_2 evolution is low at 6 LH, when synthesis of CPa and assembly of LHCP polypeptides begin (Fig. 2a). Additional dark during the whole light period decreases O_2 evolution to about 30% (Fig. 5a), whereas additional dark at 3 to 14 LH preserves high activity for an additional 2 h (Fig. 5b). Additional dark at the beginning of the light period (0–3 LH) decreases the activity of O_2 evolution for the residual light period and displaces both maxima of activity by about 3 h (Fig. 5c).

The direct connection of photosynthetic O_2 evolution and translation processes is given by experiments with cycloheximide or streptomycin (Fig. 5d). *E. gracilis* was treated with the translation-inhibitor for 1 h, and then the photosynthetic O_2 evolution and the ^{14}C incorporation into CPI, CPa, and LHCP (Fig. 6) was determined. The inhibitor of plastidial translation, streptomycin, causes strong decrease of both maxima of activity as well as inhibition of CPI and CPa synthesis (Fig. 6b), whereas the inhibitor of cytoplasmic translation, cycloheximide, effects the second maximum of activity only and inhibits LHCP-apoprotein synthesis (Fig. 6a). As reported by Ortiz and Stutz (16), polypeptides related to CPI and to CPa are synthesized in *Euglena* chloroplasts and LHCP polypeptides are synthesized in the cytoplasm. Therefore, the first maximum of activity in O_2 evolution is directly related to plastidial synthesis of CPI proteins and the second to plastidial synthesis of CPa proteins together with the assembly of LHCP proteins of cytoplasmic origin. The stage-specific synthesis of CPI in synchronized *E. gracilis* probably changes the efficiency of the photosynthetic apparatus resulting in the first maximum of O_2 evolution, whereas the stage-specific synthesis of CPa increases the amount of O_2 producing centers per cell itself and causes the second maximum of O_2 evolution. The decrease of O_2 evolution activity at 4 to 6 LH would be caused by rearrangement of CPI and beginning insertion of CPa, because O_2 evolution activity is stable for a longer time (Fig. 5b) when CPa protein synthesis is omitted (Fig. 2c).

DISCUSSION

It is of special interest to consider that, under synchronization conditions, the syntheses of PSI- and PSII-related proteins are separated in time. These results align with the reports that, in the case of greening barley seedlings, PSI activity precedes PSII activity (12, 18). Moreover, these stage-specific syntheses of thylakoid proteins in *E. gracilis* are consistent with the stage-specific changes of the activity of PSI or PSII particles (6). In particular, the activity alterations of PSI particles measured *in vitro* by methyl viologen reduction (6) as well as of PSII particles measured *in vitro* by dichlorophenolindophenol reduction (6) coincide with the *in vivo* measured changes of photosynthetic O_2 evolution in *E. gracilis*. The stage-specific decrease of the photosynthetic activities reported also for other algae (19, 23) can be explained for *E. gracilis* by the insertion of newly synthesized CPa and LHCP, both related to PSII, at 6 LH and the occurrence of new kinds of PSII particles (6) necessarily accompanied by thylakoid reorganization.

The successive insertion of CPI, CPa, and LHCP is presumably necessary for formation of an efficient photosynthetic apparatus, because simultaneous insertion (Fig. 2d) is not so efficient in photosynthetic O_2 evolution (Fig. 5c). The efficiency of O_2 evolution is therefore not only dependent on the amount of the different components of the photosynthetic apparatus but also dependent on their optimal arrangement in the thylakoid system. The regulation between synthesis of LHCP precursor proteins in the cytoplasm and their insertion simultaneous with CPa proteins into the thylakoids is beyond the scope of this paper. It should be mentioned, however, that at 6 LH the polypeptide composition and the surface pK of the chloroplast envelopes in synchronized *E. gracilis* is quite different from the other development stages (7, 8). This might be a prerequisite for the entry of LHCP precursor proteins into the Euglena chloroplasts.

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