Development of the Photosynthetic Apparatus during Light-dependent Greening of a Mutant of Chlorella fusca

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Abstract. The formation of chlorophyll, cytochrome f, P-700, ribulose bisphosphate carboxylase as well as photosynthesis and Hill reaction activities were tested during the light-dependent greening process of the Chlorella fusca mutant G 10. Neither chlorophyll nor protochlorophyllide was detected in the dark-grown cells. When transferred to light the mutant cells developed chlorophyll and established its photosynthetic capacity after a short lag phase. In the in vivo absorption spectra a spectral shift of the red absorption peak position from 674 to 680 nm was indicated during the first 3 h of greening. Cytochrome f was already present in the dark-grown cells, but during the greening phase a threefold increase in the cytochrome f content could be seen. At the early stages of greening a characteristic primary oscillation in the content of cytochrome f was observed. P-700 was lacking in the dark and during the first 30 min of illumination. From the first to the second h of light a forced synthesis of P-700 took place and the time-course curve for the ratios of P-700/chlorophyll rose to a sharp maximum. The synthesis of P-700 started together with photosystem I activity and showed similar kinetics. We found the simultaneous appearance of photosystem II, photosystem I, and photosynthetic activities 30 min after the beginning of the illumination. Based on chlorophyll content they attained maximum activity after 2 h of light, but at this time photosystem I capacity proved to be remarkably higher than photosynthetic and photosystem II activities. Highest carboxylase activity existed in dark-grown cells. During the greening process the activity of the enzyme decreased continuously. After 2 h of illumination chlorophyll synthesis partially served to increase the size of the photosynthetic unit, which consequently led to a decrease in the light energy needed to saturate photosynthesis and also to a decrease of photosynthetic rate based on chlorophyll content.

Key words: Cytochrome f — Development, photosynthetic apparatus — Greening mutant — Photochemical activities — Photosynthetic unit — P-700 — Ribulose bisphosphate carboxylase.

Introduction

The development of the photosynthetic apparatus and the appearance of photosynthetic activity have been extensively studied in higher plants and algae during the light-induced greening of previously dark-grown, etiolated organisms. The time-course of development of photochemical activity induced by illumination varies markedly with the age of the seedlings, the species of plants, and conditions of growth. It is difficult, therefore, to compare the relative times of appearance of photosynthetic activities. Most of the results clearly suggest a sequential appearance of photosystem I and photosystem II (see reviews by Park and Sane, 1971 and by Boardman, 1977). However, some results indicate that the two photosystems might by synthesized and become active at about the same time (Dodge and Whittingham, 1966; Dowdell and Dodge, 1971; Senger et al., 1975).

Since the results in higher plants and green algae are varying and different, our findings will contribute to more clarity at least in algae. The results of our studies with this mutant are adding some surprising new aspects to this problem. We have examined the formation of chlorophyll, cytochrome f, P-700,
ribulose bisphosphate carboxylase as well as photosynthesis and Hill reaction activities with benzoquinone and methylviologen as electron acceptors at different greening stages of the mutant of *Chlorella fusca* G 10, which has lost the ability to form chlorophyll in the dark.

**Materials and Methods**

The mutant G 10 (C-1.1.10.10) of *Chlorella fusca* was used (Bendix and Allen, 1962). The mutant strain differs from the wild type in not being able to synthesize chlorophyll in the dark. Cells were grown at 24°C, on a shaker in liquid medium supplemented with glucose (0.2%) for 6–7 days in the dark. The algae suspension in the Erlenmeyer flasks reached at this time a density of about 4 ml packed cell volume/l. The medium and culturing conditions were described previously (Wild and Egle, 1967; Wild et al., 1974; Bauer and Wild, 1976). For greening experiments the culture flasks were exposed to white fluorescent light of 2500 lx at the surface of the culture vessels.

The methods for the determination of chlorophyll, photosynthetic capacity, and Hill reaction activities with intact algal cells were published previously (Bauer and Wild, 1976). Photosystem II activity was measured as Hill reaction with p-benzoquinone as the oxidant. Photosystem I activity with intact algal cells was measured with methylviologen as electron acceptor and 2,6-dichlorophenolindophenol and Na-ascorbate as the electron donors. Methylviologen Hill reaction with whole cells was possible only after treatment of the cells at the different greening stages with benzoquinone (0.02–0.04%) for 15–30 min. This reagent produces an increase in the permeability of the membranes (Bauer and Wild, 1976; Gimmler 1976). O₂ evolution or O₂ uptake, respectively, was measured with a Clark-type electrode.

Cytochrome f and P-700 were determined in broken cells by the oxidized-minus-reduced difference spectra at room temperature according to Wild and Fuldner (1977) using an Aminco DW-2 spectrophotometer.

For the preparation of ribulose bisphosphate carboxylase *Chlorella* cells were harvested by centrifugation. The pelletted cells were resuspended in 20 ml tricine-KOH buffer (pH 7.5) containing 5 mmol l⁻¹ GSH, 10 mmol l⁻¹ MgCl₂, and 0.2 mmol l⁻¹ EDTA. The concentrated cell suspension was transferred to a duran glass flask 3/4 filled with a mixture of glass beads (0.2 mm and 1.0 mm in diameter) and then deep-frozen to −20°C for one day. After thawing the glass flask was vibrated for 2 min in a homogenizer (type MSK, Braun, Melsungen) while cautiously cooling with CO₂ snow. The contents of the flask were then filtered through a coarse, fritted-glass filter to remove the glass beads, which were subsequently washed with additional buffer. Cell debris was removed by centrifugation at 30,000 g and 5°C for 10 min.

The RuBP-dependent incorporation of ¹⁴CO₂ into acid-stable products was determined in a reaction mixture of 0.7 ml final volume at 24°C essentially as given by Chu and Bassham (1973). The final concentration of each component in the assay mixture was the following; 15 mmol l⁻¹ Tris-HCl (pH 7.5), 20 mmol l⁻¹ MgCl₂, 3.13 mmol l⁻¹ GSH, 0.25 mmol l⁻¹ EDTA, 0.8 mmol l⁻¹ RuBP, 21.2 mCi mol⁻¹ NaH¹⁴CO₃, 21.43 mmol l⁻¹ Na HCO₃, and 0.1 ml enzyme solution. At the end of the reaction (5 min), 0.2 ml of glacial acetic acid was added to stop the reaction. Samples were dried with warm air and the acid-stable residue redissolved in 0.4 ml H₂O. A 5-ml aliquot of scintillation cocktail was added and the samples were counted in a scintillation spectrometer.

![Absorption spectra of undiluted cell cultures of the mutant G 10 of *Chlorella* during greening (0, 1, 1.5, 2, 2.5, 3, 6, 8 h of greening). The starting culture was 6 days old and exposed to 2500 lx of white fluorescent light for greening](image-url)