ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. XXVI, 17 SECTIO C 1971

Instytut Biologii UMCS
Zakład Fizjologii Roślin

Tadeusz BASZYŃSKI, Barbara DUDZIAK,
Danuta ARNOLD

Light-stimulated Synthesis of Plastid Benzoquinones and Pigments in Cells of *Euglena gracilis* in Absence of Photosynthesis

Synteza benzochinonów i barwników plastydowych na świetle w komórkach *Euglena gracilis* w warunkach zahamowanej fotosyntezy

Синтез пластидовых бензохинонов и красителей на свету в клетках *Euglena gracilis* в условиях замедленного фотосинтеза

INTRODUCTION

The synthesis of plastid quinones and chloroplast pigments is stimulated by light (quot. 11). There is, however, not enough evidence to prove direct relationship between this process and photosynthesis. Previous work from this laboratory (3) has stated that synthesis of α-tocopherol (α-T) in light-grown cells of *Euglena gracilis* is independent of light intensity. This fact seems to exclude the participation of photosynthesis in α-T synthesis and to promote the suggestion that photosynthetic competence is not necessary for α-T synthesis in *Euglena gracilis* cells.

Taking into account this suggestion, the authors examined the formation of benzoquinones and pigments in non-dividing *Euglena gracilis* cultures treated with DCMU (3,4-dichlorophenyl-N,N-dimethylurea) as a known inhibitor of electron flow from system II of photosynthesis.

MATERIAL AND METHODS

Cells of *Euglena gracilis*, strain Z, from the Museum of Cultures of Autotrophic Organisms in Prague, were used. They were cultured on Pringsheim and Pringsheim medium (12) in darkness, in flasks wrapped up in aluminium

foil at room temperature for six days. Then they were collected by centrifuging and transferred to resting medium (15). All examinations were carried out on non-dividing cells.

Such quantities of DCMU were added to 350 ml of resting medium so as to obtain the final concentration 10^{-5} M (ethanol concentration in the medium was then 0.1% and such an amount of pure ethanol was added to control cultures). Centrifuged *Euglena gracilis* cells were inoculated in flasks so that their number in 1 ml of the medium did not exceed 10^6. The cultures were then put on a shaker in the light, the intensity of which was about 2000 lx.

At definite time intervals (after 0, 1, 3, 5, 7 days since exposure to light) the content of plastoquinone (PQ), α-tocopherolquinone (α-TQ) and its chromanol, α-tocopherol, chlorophyll and β-carotene was determined in the cells of *Euglena gracilis*. Quinones and β-carotene were determined according to Threlfall and Goodwin (16), whereas chlorophyll according to Arnon (1).

To check the inhibition of photosynthesis by DCMU, photosynthetic activity of control and DCMU-treated cells was measured in microrespirometer (18). This method seemed to be useful because DCMU inhibits completely both CO_2-fixation and photosynthetic oxygen evolution (4).

RESULTS AND DISCUSSION

The starting-point for these experiments was the finding of Schiff and his co-workers (13) that in *Euglena gracilis cells* DCMU inhibits photosynthetic CO_2-fixation and it does not effect significantly the formation of chlorophyll and light induced chloroplast development. Complete inhibition of $^{14}CO_2$-fixation by DCMU in suspension of *Chlorella* was found by Zweig and his co-workers (quot. 17). The concentration of DCMU applied in our experiments also caused complete inhibition of photosynthetic oxygen evolution. Due to that it was possible to study the participation of light in synthesis of benzoquinones and plastid pigments independently of photosynthesis as a source of substrates. Although DCMU slightly changes the structure of plastids, their photosynthetic competence is recovered after washing out the inhibitor. Changes in the internal structure of plastids were also observed during CMU (3-(p-chlorophenyl)-1,1-dimethylurea) action on higher plants which resulted in number decrease of grana with simultaneous increase of their size and of the amount of thylakoids contained in them (10). As concentration and synthesis of chloroplastidic quinones and pigments are related to the degree of chloroplast development (2), partial inhibition of the synthesis of these compounds caused by DCMU effect on plastid structure had to be taken into account. It appeared, however, that the effect mentioned made it possible to observe the dependence of quinone biosynthesis on photosynthesis.

Our results concerning chlorophyll accumulation during 7 days of growth of dark-grown resting cells in the light confirm the data of

Schiff and his co-workers (13), whereas inhibition of chlorophyll biosynthesis was already observed after the first day of illumination (Fig. 1). Inhibition percentage was a little higher than that found by the authors quoted above but it never exceeded 40%. In view of complete inhibition of photosynthesis under these conditions chlorophyll biosynthesis seems to be independent of photosynthetic competence of cells but related to the change of plastid structure caused by DCMU. The independence of those processes can also be supported by earlier observations (10) that in leaves of higher plants CMU (10^{-4} M) completely inhibits photosynthesis, whereas chlorophyll accumulation after 48 hrs only in about 50—60%. In the opinion of the above authors,

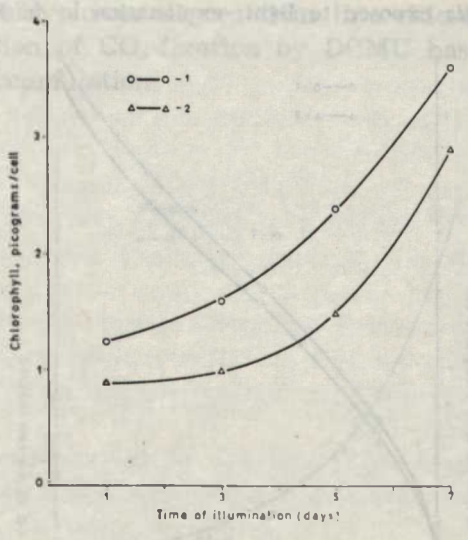

Fig. 1. Chlorophyll synthesis by dark-grown resting cells of *E. gracilis* exposed to light in the presence or absence of photosynthesis; 1 — control (green) cells; 2 — DCMU-treated cells

inversion of this inhibition by saccharose may prove that the role of photosynthesis does not seem to be specific here.

The formation of β-carotene is rapid during greening up of dark-grown *Euglena* cells. In our experiments fifteen-fold increase of β-carotene content was observed after 7 days of illumination (Fig. 2). According to Goodwin and Jamicorn (quot. 7) *Euglena gracilis*, var. *bacillaris* produces five times more carotenoids in the light than in the dark under the same conditions. The inhibition of β-carotene synthesis induced by DCMU in our case was, at the end of experiment, about 13%; thus it can be assumed that it was brought about by partial disturbance in chloroplast development. It does not seem that this

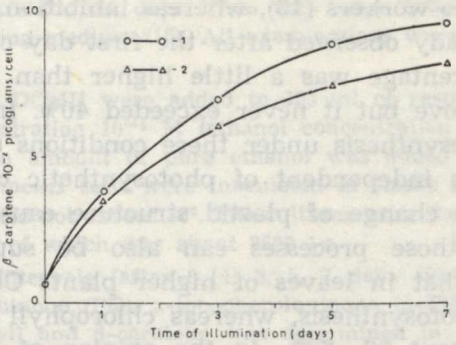

Fig. 2. Effect of DCMU on β-carotene synthesis by dark-grown resting cells of *E. gracilis* exposed to light; explanation as in Fig. 1

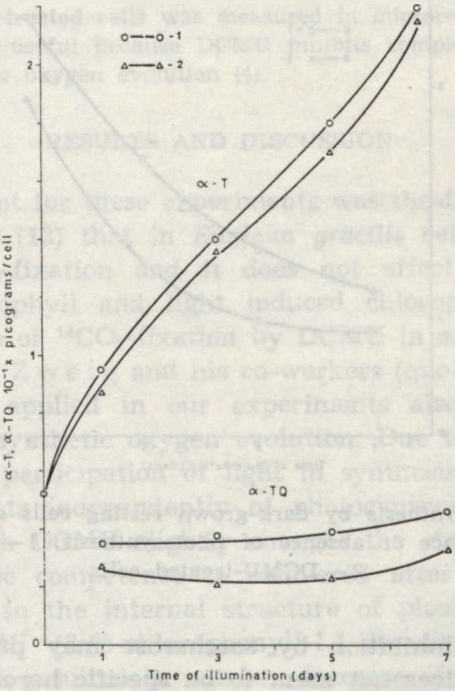

Fig. 3. Synthesis of α-tocopherol and α-tocopherolquinone by dark-grown resting cells of *E. gracilis* exposed to light; explanation as in Fig. 1

inhibition might depend to some extent on photosynthesis. At present no data on the influence of photosynthesis inhibitors on β-carotene formation are available. Goodwin (8) reported about inhibition of carotenoids synthesis by removal of CO_2 in higher plants. However, the same author found that $^{14}CO_2$ was not incorporated into β-carotene by *Phycomyces blakesleeanus* growing in a special medium (7).

Results concerning α-T support the suggestion which was expressed earlier (3), that its synthesis is independent of photosynthesis. The curves presented in Fig. 3 show that DCMU does not effect α-T level in cells at all. It was already reported by B u c k e (5), when attempting to determine the condition in which α-T is oxidized to α-TQ by chloroplast from broad bean leaves, that such inhibitors of photosynthesis as CMU and salicylaldoxime had no effect on the endogenous α-T level in chloroplast. Photosynthetic independent synthesis of α-T finds support in the studies of G r i f f i t h s and his co-workers (9) on incorporation of $^{14}CO_2$ into terpenoid quinones and related compounds by tobacco seedlings. It appears from the studies that $^{14}CO_2$ is incorporated into α-T at a considerably lower degree than into chloroplastidic quinones. Hence the inhibition of CO_2-fixation by DCMU has no significant influence on α-T accumulation.

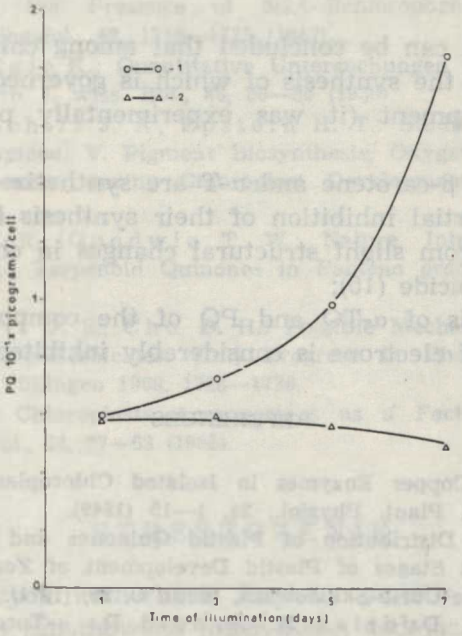

Fig. 4. Effect of DCMU on plastoquinone synthesis by dark-grown resting cells of *E. gracilis* exposed to light; explanation as in Fig. 1

The formation of α-TQ and PQ in the time of illumination of etiolated non-dividing *Euglena cells* coincides with the studies of T h r e l f a l l and G o o d w i n (16). In the process of greening up of cells the increase of PQ amount is particularly rapid (Fig. 4). This fact supports L i c h t e n t h a l e r ' s hypothesis (11) that white light primarily

promotes the synthesis of those plastid lipids that are present in etiolated plants in relatively low concentrations. DCMU inhibits the accumulation both of PQ and α-TQ that of the former, however, at considerably higher a degree.

When assuming that a high level of quinones in illuminated cells results from the shift of equilibrium in oxidized form (such possibility was found by Dilley and Crane (6) in isolated chloroplasts of spinach) the inhibitory action of DCMU could consist in a disturbance of this equilibrium. The evidence for the relationship between photooxidation and photosynthetic transport of electrons is among others the inhibition of light-induced changes in quinone level by o-phenantroline.

On the other hand, much greater percentage of $^{14}CO_2$ was present in the PQs (9) during incorporation of $^{14}CO_2$ into prenyl portion of terpenoid quinone by tobacco seedlings. Hence the inhibition of PQs synthesis can be induced by the inhibition of CO_2-fixation in DCMU treated cells.

Summing up it can be concluded that among chloroplastidic quinones and pigments, the synthesis of which is governed by the degree of chloroplast development (it was experimentally proved in another paper (2)):

1) chlorophyll, β-carotene and α-T are synthetized irrespectively of photosynthesis (partial inhibition of their synthesis induced by DCMU probably results from slight structural changes in chloroplasts brought about by this herbicide (15);

2) the synthesis of α-TQ and PQ of the compounds participating in the transport of electrons is considerably inhibited by DCMU.

REFERENCES

1. Arnon D. I.: Copper Enzymes in Isolated Chloroplast. Polyphenoloxidase in *Beta vulgaris*. Plant. Physiol., 24, 1—15 (1949).
2. Baszyński T.: Distribution of Plastid Quinones and Pigments along the Leaf in Different Stages of Plastid Development of *Zea mays* L. Seedlings. Ann. Univ. Mariae Curie-Skłodowska, sectio C, 26, (1971).
3. Baszyński T., Dudziak B., Arnold D.: α-Tocopherol Synthesis in Streptomycin-treated Cells of *Euglena gracilis*. Ann. Univ. Mariae Curie-Skłodowska, sectio C, 24, 1—8 (1969).
4. Bishop N. I.: Influence of the Herbicide, DCMU, on the Oxygen-evolving System of Photosynthesis. Biochim. Biophys. Acta, 27, 205—206 (1958).
5. Bucke C.: On the Interconversion of α-Tocopherol and α-Tocopherolquinone in Broad Bean Chloroplasts. [In:] Progress in Photosynthesis Research. Ed. H. Metzner, Tübingen 1969, 325—331.
6. Dilley R., Crane F. L.: Light-dependent Conversions of Endogenous α-Tocopherolquinone and Plastoquinone-D in *Spinacia oleracea* Chloroplasts. Plant Physiol., 39, 33—36 (1964).

7. Goodwin T. W.: The Biosynthesis and Function of Carotenoid Pigments. Advances in Enzymology. Ed. F. F. Nord, Interscience Publishers Inc., New York 1959, **21**, 295—368.
8. Goodwin T. W.: The Biosynthesis of Vitamins and Related Compounds. Academic Press, London and New York 1963, 292.
9. Griffiths W. T., Threlfall D. R., Goodwin T. W.: Observations on the Nature and Biosynthesis of Terpenoid Quinones and Related Compounds in Tobacco Shoots. European J. Biochem., **5**, 124—132 (1968).
10. Klein S., Neuman J.: The Greening of Etiolated Bean Leaves and the Development of Chloroplast Fine Structure in Absence of Photosynthesis. Plant and Cell Physiol., **7**, 115—123 (1966).
11. Lichtenthaler H. K.: Light-stimulated Synthesis of Plastid Quinones and Pigments in Etiolated Barley Seedlings. Biochim. Biophys. Acta, **184**, 164—172 (1969).
12. Pringsheim E. G., Pringsheim O.: Experimental Elimination of Chromatophores and Eye-Spot in *Euglena gracilis*. New Phytol., **51**, 65—76 (1952).
13. Schiff J. A., Zeldin M. H., Rubman J.: Chlorophyll Formation and Photosynthetic Competence in *Euglena* during Light-induced Chloroplast Development in the Fresence of 3(3,4-dichlorophenyl)1,1-Dimethyl Urea (DCMU). Plant Physiol., **42**, 1716—1725 (1967).
14. Seybold A., Egle K.: Quantitative Untersuchungen über Chlorophyll und Carotenoide. Jahrb. f. wiss. Bot., **86**, 50—80 (1938).
15. Stern A. J., Schiff J. A., Epstein H. T.: Studies of Chloroplast Development in *Euglena*. V. Pigment Biosynthesis, Oxygen Evolution and Carbon Dioxide Fixation during Chloroplast Development. Plant Physiol., **39**, 2, 220—226 (1964).
16. Threlfall D. R., Goodwin T. W.: Nature, Intracellular Distribution and Formation of Terpenoid Quinones in *Euglena gracilis*. Biochem. J., **103**, 573—588.
17. Zweig G., Hitt J. E., Cho D. H.: Possible Mechanism of the Mode of Action of Quinone-Herbicides. [In:] Progress in Photosynthesis Research. Ed. H. Metzner, Tübingen 1969, 1728—1736.
18. Zurzycki J.: Chloroplasts Arrangement as a Factor in Photosynthesis. Acta Soc. Bot. Fol., **24**, 27—63 (1955).

STRESZCZENIE

W spoczynkowych komórkach *Euglena gracilis*, inkubowanych na świetle, w których zahamowano fotosyntezę, dodając do podłoża DCMU, badano syntezę benzochinonów i barwników plastydowych. Zebrane w określonych odstępach czasu kultury *Euglena gracilis* ekstrahowano i rozdzielano na poszczególne frakcje lipidowe metodą chromatografii kolumnowej wg Threlfalla i Goodwina (16). Oznaczano w nich zawartość chlorofilu, β-karotenu, plastochinonu, α-tokoferolochinonu oraz α-tokoferolu.

Stwierdzono, że chlorofil, β-karoten i α-tokoferol są syntetyzowane w komórkach *Euglena gracilis* niezależnie od fotosyntezy. Częściowa in-

hibicja ich syntezy jest prawdopodobnie wynikiem nieznacznych zmian w strukturze chloroplastów wywołanych przez DCMU.

Synteza plastochinonu i α-tokoferolochinonu, związków biorących udział w transporcie elektronów, jest w znacznym stopniu inhibowana przez DCMU.

РЕЗЮМЕ

В неразмножающихся клетках *Euglena gracilis*, инкубированных на свету, в которых замедлили фотосинтез, добавляя в питательную среду DCMU, исследовали синтез пластидовых бензохинонов и красителей. Собранные в определенные промежутки времени культуры *Euglena gracilis* перколировали и разделили на отдельные липидовые фракции методом хроматографии на колонне по Трельфаллю и Гудвину (16). В них определяли содержание хлорофилла, β-каротина, пластохинона, α-токоферолохинона и α-токоферола.

Констатировали, что хлорофилл, β-каротин и α-токоферол синтезируются в клетках *Euglena gracilis* независимо от фотосинтеза. Частичное сдерживание их синтеза является, правдоподобно, результатом незначительных изменений в структуре хлоропластов вызванных DCMU.

Синтез пластохинона и α-токоферолохинона, соединений, участвующих в транспорте электронов, в значительной степени задерживается DCMU.

Pap. druk. sat. III kl., 80 g Format B5 (70×100) Stron druku: 8
Annales UMCS, Lublin 1971 Drukarnia Uniwersytecka w Lublinie Zam. nr 12 z dnia 18.I.1971
950+50 egz. A-7 Maszynopis otrzymano 18.I.1971 Druk ukończono 25.VIII.71