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α-Tocopherol Synthesis in Streptomycin-Treated Cells of *Euglena gracilis*

Synteza α-tokoferolu w komórkach *Euglena gracilis* poddanych działaniu streptomycyny

Синтез α-токоферола в клетках *Euglena gracilis*, находящихся под действием стрептомицина

INTRODUCTION

Tocopherols occur both in photo- and nonphotosynthetic tissues. Among vitamin E active compounds, α-tocopherol (α-T) is chiefly present in the green parts of plants. Other tocols and tocotrienols occur rather in the chloroplast-free plant organs. Dilley and Crane (7) in their investigations on subcellular distribution of α-T in the spinach and lilac leaf tissues showed that chloroplast fraction of a homogenized plant tissue contained almost the whole pool of α-T present in the cell, whereas other fractions contained it only in trace quantities.

Goodwin and Mercer's studies (12) of the incorporation of ^{14}C-labelled mevalonic acid lactone and $^{14}CO_2$, during greening process of some etiolated tissues, led them to assume that α-T was synthesized in chloroplasts. However, α-T is known to appear or to increase its participation in the total of tocols (2, 3, 11) in seeds during germination prior to formation of chloroplast. On this basis α-T was supposed to be formed partly by transmethylation of mono- and dimethyl derivates. This hypothesis was recently confirmed by Threlfall and his coworkers (26) who demonstrated the incorporation of CH_3 into the chromanol ring. However, the synthesis of α-T is only possible until α-T

precursors have been utilized. According to Hall and Laidman (14) the formation of α-T during seed germination by methylation of dimethyl tocopherols of the resting grain can only partly increase α-T.

The greatest amount of α-T is known to be found in the green plant tissues. Hence, α-T synthesis is assumed to depend on light. The light-dependent nature of α-T synthesis is disputed by Gaunt and Stowe (11) according to whom no changes have been found in the α-T level during greening of etiolated pea leaves. Threlfall and Griffiths (25) and Hall and Laidman (14) also found considerably smaller variations in the amount of α-T, during greening of etiolated maize and wheat seedlings, than those observed in the amount of chloroplastidic isoprenoid compounds.

The purpose of the present paper is to check whether α-T synthesis on the cell occurs simultaneously with chlorophyll synthesis due to light-induced development of the chloroplast fine structure or whether it is independent of the latter.

Studies were carried out of the cells of the *Euglena gracilis*, strain Z, which is capable of living both under auto- and heterotrophic conditions. *Euglena gracilis* was chosen because proplastides did not form chloroplasts under the influence of streptomycin which inhibits light-induced chlorophyll accumulation (19, 20). Although higher plants react similarly to streptomycin (8, 9, 10, 21), they are not able to live under heterotrophic conditions.

Recently, intracellular distribution and formation of terpenoid quinones in *Euglena gracilis* (24) were studied. In these studies the problem of α-tocopherylquinone and its parent chromanol was dealt with, but no answer has been given whether the α-T synthesis was induced by light irrespective of the development of the chloroplast fine structure.

The present paper deals with the synthesis in the light-grown cells of *Euglena gracilis* in which the development of chloroplasts was inhibited by streptomycin. The dynamics of α-T and chlorophyll in the etiolated and autotrophic cultures was observed for comparison purposes.

MATERIAL AND METHODS

Cells of *Euglena gracilis*, strain Z, from the Museum of Cultures of Autotrophic Organisms, in Prague, were used. Experiments were carried out on Pringsheim and Pringsheim medium (17) (in a thermostate) at 21 ± 1°C. The cells were grown in 350 ml of medium in 1,000 ml Erlenmayer flasks. The etiolated cells were grown in darkness in flasks wrapped in aluminium foil. The light-grown cells were illuminated by "day light" fluorescent tubes. In the experiments the following light intensities were used: about 2,000 lux promoting chloroplast development and being optimal for chlorophyll formation (22); and about 24,000 lux required for satura-

tion of photosynthesis (23). Calculations of ft-c into lux were made according to the formula given by Rabinowitch (18). Experimental cultures were obtained by inoculating the medium with an active 4-day-old etiolated liquid culture. All the cultures were shaken vigorously for 30 sec. twice a day.

To examine the effect of streptomycin on the synthesis of α-T and chlorophyll, the etiolated cells were transferred into the medium containing streptomycin (500 μg/ml) in such a quantity as to reach the end count of 10^6/ml. The flasks were kept in darkness for 7 days and next they were exposed to light and their contents were examined at time intervals (see Figures). The cells were harvested by low-speed centrifugation. The collected material was washed twice with distilled water and centrifuged. The *Euglena gracilis* cells were examined for α- content by the method of Booth (4). Chlorophyll content ($a + b$) was determined by the method of Arnon (1). The data of α-T and chlorophyll contents were expressed in picograms per 1 cell of *Euglena gracilis*. The cell number in the medium was estimated in Bürker's chamber. Dry weight of the harvested cells was determined at 105°C.

RESULTS AND DISCUSSION

In the experiments on the synthesis of α-T in the cells of *Euglena gracilis*, the development of chloroplasts was determined by chlorophyll accumulation. The changes in the amount of chlorophyll presented in Fig. 1 agree with Rosen and Gawlik's observations (19) about a rapid increase of chlorophyll synthesis in the initial greening stages

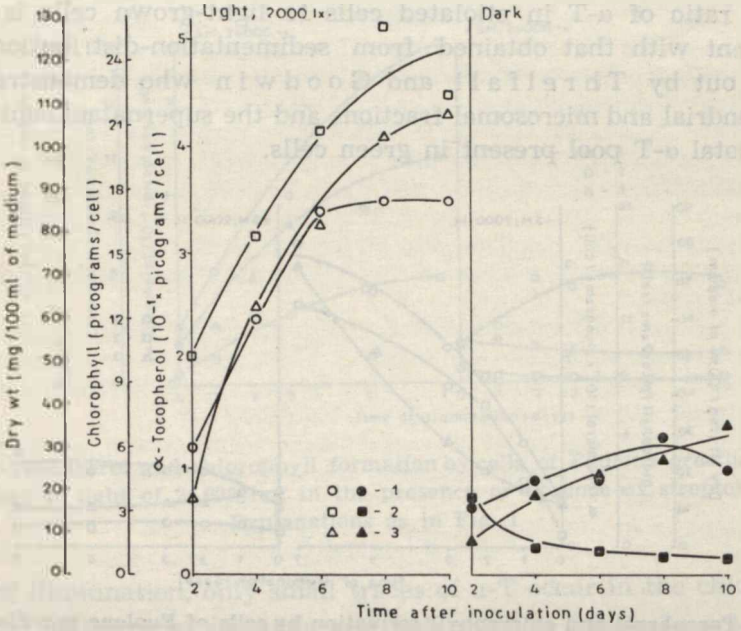

Fig. 1. The synthesis of α-tocopherol and chlorophyll by cell of *Euglena gracilis*, strain Z, grown in light or dark conditions, in relation to age. 1 — α-tocopherol, 2 — chlorophyll, 3 — dry weight of cells

of the etiolated cells. The rate of chlorophyll accumulation stabilized after 6—8 days.

The amount of the chlorophyll accumulated in the cells depended on the intensity of light used for illumination of the cells. At 2,000 lux, considered to be the optimal intensity for chloroplast development and chlorophyll accumulation (23), the results were twice higher than those obtained at 24,000 lux.

In light-grown cells treated with streptomycin, the chlorophyll content was considerably lower than that in light-grown controls and the difference increased with time of illumination. The reason of the increase was the fact that no chlorophyll formation was observed in streptomycin--treated cells. As demonstrated by Rosen and Gawlik (19), the concentration of streptomycin used in our experiments inhibited completely the development of chloroplast precursors into a functional chloroplast and light-induced chlorophyll accumulation. α-T occurs in dark- and light-grown cells of *Euglena gracilis*. Although α-T is known to occur only in chloroplasts (trace quantities of other derivates were disregarded (7), fairly considerable amounts of α-T were found in dark--grown etiolated cells (Fig. 1). The presence of α-T in the etiolated cells confirms the observations of Threlfall and Goodwin (24) that α-T is also associated with structures different than chloroplasts.

The ratio of α-T in etiolated cells to light-grown cells is in good agreement with that obtained from sedimentation-distribution studies carried out by Threlfall and Goodwin who demonstrated that mitochondrial and microsomal fractions and the supernatant contained 1/3 of the total α-T pool present in green cells.

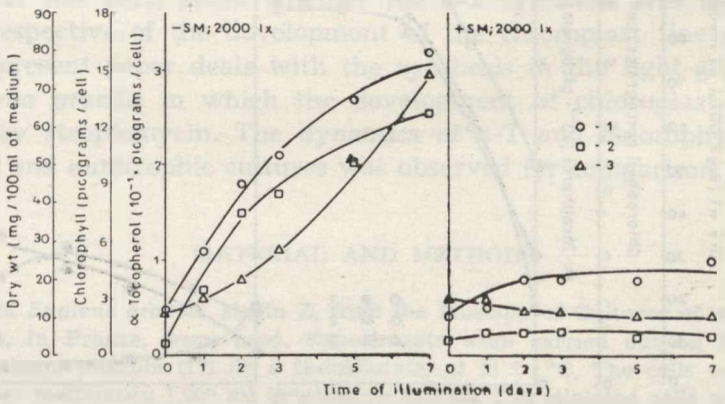

Fig. 2. α-Tocopherol and chlorophyll formation by cells of *Euglena gracilis*, strain Z, exposed to light of 2,000 lux in the presence or abscence of streptomycin. Explanations as in Fig. 1

It is worth mentioning that in etiolated higher plants α-T can also come from numerous osmophilic plastoglobuli present in thylakoid free proplastides, which, according to Lichtenthaler (16), are reservoirs of lipoquinones. Moreover, although α-T is the principal isomer in green leaves, Booth (5) found mono- and dimethyltocols in cells outside chloroplasts in *Taxus baccata* and *Hedera helix*.

Our studies of 5—7-day old cultures showed the occurrence of some amounts of γ-T in the cells of *Euglena gracilis* grown at 24,000 lux.

The synthesis of α-T in the light-grown cells in all three experimental combinations is similar and equal to chlorophyll synthesis, i.e. up to the development of the chloroplast fine structure (Figs. 1—3). The dynamics of α-T in the dark-grown and streptomycin-treated cells did not undergo any greater changes in the course of studies and the curves were similar in both cases. Thus it is assumed that α-T synthesized in the extra chloroplastidic part of the cell is independent of light. The content of α-T in the streptomycin-treated cells was considerably smaller when compared with that in the light-grown green cells. The reduction of α-T synthesis in the streptomycin-treated cells in relation to the control ones seems to indicate that light does not induce this synthesis if the cell is deprived of mature chloroplasts. This is also evidenced by the fact that indepen-

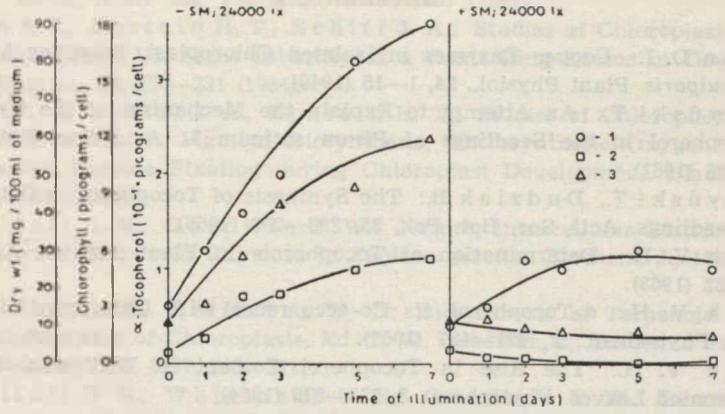

Fig. 3. α-Tocopherol and chlorophyll formation by cells of *Euglena gracilis*, strain Z, exposed to light of 24,000 lux in the presence or absence of streptomycin. Explanations as in Fig. 1

dently of illumination, only small traces of α-T occur in the chlorophyll-free part of the *Acer negundo* and *Pelargonium zonale* leaves in comparison with their green part (15).

The increase of light intensity required for saturation of photosynthesis (Fig. 3) in experiment III aimed at obtaining some preliminary information concerning a possible participation of photosynthesis in the α-T formation. The level of α-T being independent of light intensity seems to exclude the participation of photosynthesis in supplying precursors, although the ratio of α-T to chlorophyll is different from that in other experiments.

The present studies showed the necessity of the well developed chloroplast fine structure for the synthesis of the main quantity of α-T. The α-T synthesis in leaves, as demonstrated by Booth (6), can also take place in darkness in the presence of mature chloroplasts. The necessity of a suitable chloroplast structure is also stressed by Goodwin (13) in his attempt to explain the regulation of terpenoid synthesis in chloroplasts of high plants by an enzyme segregation and specific permeability of the plastid mebrane to melavonic acid.

The role of light should thus be limited to the formation of the chloroplast structure indispensable for the synthesis of its components, including α-T. Taking all this into consideration, the hypothesis of Gaunt and Stowe (11) and that of Threlfall and Griffiths (25) on the light independent nature of α-T synthesis seems to be reasonable.

REFERENCES

1. Arnon D. I.: Copper Enzymes in Isolated Chloroplast. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol., **24**, 1—15 (1949).
2. Baszyński T.: An Attempt to Explain the Mechanism of the Synthesis of α-Tocopherol in the Seedlings of *Pisum sativum* L. Acta Soc. Bot. Pol., **30**, 307—326 (1961).
3. Baszyński T., Dudziak B.: The Synthesis of Tocopherol in Cotyledonless Pea Seedlings. Acta Soc. Bot. Pol., **35**, 293—299 (1966).
4. Booth V. H.: Determination of Tocopherols in Plant Tissues. Analyst, **88**, 627—632 (1963).
5. Booth V. H.: α-Tocopherol, its Co-occurrence with Chlorophyll in Chloroplasts. Phytochem., **2**, 421—427 (1963).
6. Booth V. H.: The Rise in Tocopherol Content in Wilting and in Non-illuminated Leaves. Phytochem., **3**, 273—276 (1964).
7. Dilley R. A., Crane F. L.: Subcellular Distribution of α-Tocopherol in Spinach and Lilac Leaf Tissue. Plant Physiol., **38**, 452—456 (1963).
8. Deken-Grenson M. de: Action de la streptomycine sur la formation des chloroplastes. Biochim. Biophys. Acta, **17**, 35—47 (1955).
9. Döbel P.: Untersuchungen der Wirkung von Streptomycin-, Chloramphenicol- und 2-Thiouracil-behandlung auf die Plastidenentwicklung von Lycopersinum esculentum. Biol. Zentralblatt, **82**, 275—295 (1963).
10. Euler H. v.: Einfluss des Streptomycins auf die Chlorophyllbildung. Kem. Arb. N. F., **2**, 9—21 (1947).

11. Gaunt J. K., Stowe B. B.: Analysis and Distribution of Tocopherols and Quinones in the Pea Plant. Plant Physiol., **42**, 851—858 (1967).
12. Goodwin T. W., Mercer E. I.: The Regulation of Sterol and Carotenoid Metabolism in Germinating Seedlings [w:] The Control of Lipid Metabolism. Biochem. Soc. Symp., **24**, Ed. J. K. Grant, Academic Press, London 1963, 37—41.
13. Goodwin T. W.: Terpenoids and Chloroplast Development [w:] Biochemistry of Chloroplasts, Ed. T. W. Goodwin., **2**, Academic Press, London and New York 1967, 721—733.
14. Hall G. S., Laidman D. L.: The Pattern and Control of Isoprenoid Quinone and Tocopherol Metabolism in the Germinating Grain of Wheat (*Triticum vulgare*). Biochem. J., **108**, 475—482 (1968).
15. Lichtenthaler H. K.: Verbreitung und Konzentration des α-Tocopherols in Chloroplasten. Ber. Dtsch. Bot. Ges., **79**, 111—117 (1966).
16. Lichtenthaler H. K.: Beziehung zwischen Zusammensetzung und Struktur der Plastiden in grünen und etiolierten Keimlingen von *Hordeum vulgare* L. Z. Pflanzenphysiol., **56**, 273—281 (1967).
17. Pringsheim E. G., Pringsheim O.: Experimental Elimination of Chromatophores and Eye-Spot in *Euglena gracilis*. New Phytol., **51**, 65—76 (1952).
18. Rabinowitch E. I.: Photosynthesis and Related Processes. Intersc. Publ. Inc. New York 1951.
19. Rosen W. G., Gawlik S. R.: Effect of Streptomycin on Chlorophyll Accumulation in *Euglena gracilis*. Jour. Protozool., **8**, 90—96 (1961).
20. Sigesmund K. A., Rosen W. G., Gawlik S. R.: Effects of Darkness and of Streptomycin on the Fine Structure of *Euglena gracilis*. Amer. Jour. Bot., **49**, 137—145 (1962).
21. Signol M.: Comparaison de l'action de la dihydrostreptomycine à celle de l'acide 3-(α-iminoéthyl)-5-methyltétronique sur l'infrastructure des chloroplastes. Compt. Rend. Acad. Sci. Paris, **27**, 1993—1995 (1961).
22. Stern A. I., Epstein H. T., Schiff J. A.: Studies of Chloroplast Development in *Euglena*. VI. Light Intensity as a Controlling Factor in Development. Plant Physiol., **39**, 226—231 (1964).
23. Stern A. I., Schiff J. A., Epstein H. T.: Studies of Chloroplast Development in *Euglena*. V. Pigment Biosynthesis, Photosynthetic Oxygen Evolution and Carbon Dioxide Fixation during Chloroplast Development. Plant Physiol., **39**, 220—226 (1964).
24. Threlfall D. R., Goodwin T. W.: Nature, Intracellular Distribution and Formation of Terpenoid Quinones in *Euglena gracilis*. Biochem. J., **103**, 573—588 (1967).
25. Threlfall D. R., Griffiths W. T.: Biosynthesis of Terpenoid Quinones [w:] Biochemistry of Chloroplasts, Ed. T. W. Goodwin., **2**, Academic Press, London and New York 1967, 255—271.
26. Threlfall D. R., Whistance G. R., Goodwin T. W.: Biosynthesis of Phytoquinones. Incorporation of L- (Me- ^{14}C, ^{3}H) Methionine into Terpenoid Quinones and Chromanols in Maize Shoots. Biochem. J. **106**, 107—112 (1968).

STRESZCZENIE

Badano wpływ streptomycyny na syntezę α-tokoferolu oraz akumulację chlorofilu w komórkach *Euglena gracilis*. Kultury rosły na podłożu Pringsheima i Pringsheima na świetle o intensywności 1°, optymalnej dla

rozwoju chloroplastów i akumulacji chlorofilu, oraz 2°, wymaganej dla nasycenia fotosyntezy. α-Tokoferol oznaczano metodą Bootha. Chlorofil ($a + b$) określano według Arnona.

Stwierdzono, że synteza α-tokoferolu jest możliwa jedynie w obecności dojrzałych chloroplastów i do wykształcania struktury chloroplastów ogranicza się rola światła. Przedstawiono wstępne dane, wykluczające udział fotosyntezy. Wykazano, że α-tokoferol, obecny w pozachloroplastowej części komórki, nie ulega istotnej zmianie pod względem światła.

РЕЗЮМЕ

Исследовались влияние стрептомицина на синтез α-токоферола и на аккумуляцию хлорофилла в клетках *Euglena gracilis*. Культуры росли на субстрате Pringsheima и Pringsheima на свету интенсивности 1°, оптимального для развития хлоропластов и аккумуляции хлорофилла, а также на свету интенсивности 2°, необходимом для насыщения фотосинтеза.

α-токоферол определялся по методу Буса, а хлорофилл ($a+b$) — по методу Арнона.

Установлено, что синтез α-токоферола возможен только в присутствии зрелых хлоропластов и этим ограничивается роль света. Приводятся предварительные данные, исключающие участие фотосинтеза. Установлено, что α-токоферол, присутствующий во внехлоропластовой части клетки, под действием света изменяется несущественно.

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