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Barbara DUDZIAK, Zbigniew KRUPA,
Tadeusz BASZYŃSKI

Effect of Chloramphenicol on the Synthesis of Plastid Benzoquinones and Pigments in Greening Cells of *Euglena gracilis*

Wpływ chloramfenikolu na syntezę plastydowych benzochinonów i barwników w zieleniejących komórkach *Euglena gracilis*

Влияние хлорамфеникола на синтез пластидных бензохинонов и красителей в зеленеющих клетках *Euglena gracilis*

INTRODUCTION

Chloramphenicol is a powerful inhibitor of peptide bond formation in bacteria (8) and has proved to be an excellent tool for study of cell processes because of its selective locus of action. It binds to the 70 S species of ribosomes present in bacteria, blue-green algae, and organelles such as plastids and mitochondria of plant and animal cells (2, 9, 34). Because of its ability to suppress protein synthesis without affecting cytoplasmic protein synthesis chloramphenicol has been increasingly used in studies of plastid and mitochondrial development.

Chloramphenicol has been shown to inhibit plastid protein synthesis, especially light-induced synthesis of electron transfer proteins in dark-grown *Euglena* cells (28), fraction I protein in etiolated leaves (20, 23) and dark-grown *Euglena* cells (28), and the synthesis of nitrite reductase in green corn leaves (27). It is also an inhibitor of plastid lipid synthesis (7).

Light-induced synthesis of chlorophyll is blocked by chloramphenicol in etiolated bean leaves (10, 19, 21, 22) and dark-grown *Euglena* cells (1, 18, 25, 28).

Kirk (15) and Nikołajewa et al. (24) have also shown the inhibition of β-carotene synthesis.

The authors examined the formation of plastid benzoquinones and pigments in non-dividing *Euglena gracilis* treated with chloramphenicol.

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 (26) in darkness, in flasks wrapped up in aluminium foil at room temperature for six days. Then they were collected by centrifugation and transferred to resting medium (30). All experiments were carried out on non-dividing cells. The centrifuged *Euglena gracilis* cells were inoculated in flasks so that their number did not exceed 10^6 in 1 ml of the medium. Chloramphenicol was added in the concentration of 1 mg per 1 ml of the medium according to Kirk (15) and Nikołajewa et al. (24).

The cultures were then put on a shaker in the light, the intensity of which was about 2000 lx. 0, 1, 2, 3, 4, 5, 6, 7 days after the exposure to light the content of plastoquinone A (PQA), reduced plastoquinone A ($PQAH_2$), α-tocopherolquinone (α-TQ) and its chromanol α-tocopherol (α-T), chlorophyll, β-carotene, and xantophylls (antheraxanthin and neoxanthin) were determined in the cells of *Euglena gracilis*.

Quinones were determined according to Lichtenthaler (17), whereas carotenoids according to Hager and Bertenrath (14). The *Euglena gracilis* cells were examined for chlorophyll content by the method of Arnon (3). All results were calculated from three replicas.

RESULTS AND DISCUSSION

Our experiments on the synthesis of plastid benzoquinones and pigments in greening cells of *Euglena gracilis* showed that chloramphenicol is a strong inhibitor of the synthesis of chloroplast compounds. It appeared that chloramphenicol is not only the inhibitor of chlorophyll and β-carotene formation (1, 10, 15, 18, 21, 22, 24, 25) but also of PQA, $PQAH_2$, α-TQ, α-T and xantophyll (antheraxanthin and neoxanthin) synthesis.

It is well known that light induces the development of chloroplasts and formation of plastid lipoquinones and pigments. Chloroplast development is determined by chlorophyll accumulation. A rapid increase of chlorophyll synthesis in the initial greening stages of the etiolated non-dividing *Euglena gracilis* cells (Fig. 1) as shown by us, was also observed by other authors (4, 5, 7, 15).

In dark-grown cells exposed to light in the presence of chloramphenicol, the chlorophyll content was lower than in dark-grown cells exposed to light in the absence of chloramphenicol (Fig. 1). The inhi-

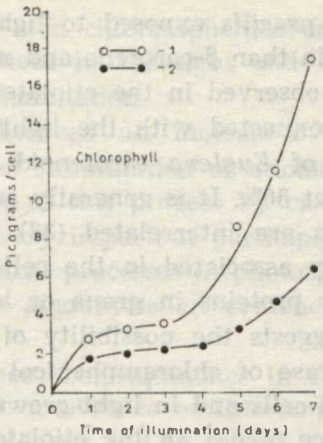

Fig. 1. Chlorophyll formation by dark-grown cells of *Euglena gracilis*, strain Z, exposed to light in the presence or absence of chloramphenicol (CAM); 1 — control (green) cells; 2 — CAM-treated cells

bition of chlorophyll biosynthesis was observed after the first day of illumination. The inhibition percentage was about 40—65% and therefore similar to that obtained by K i r k (15) and N i k o ł a j e w a et al. (24).

The significant aspect of the plastid carotenoids of *Euglena gracilis* is that apart from the main xantophyll they are the same as those in the green algae; antheraxanthin is known to be the main xantophyll (12). There are also other carotenoids like β-carotene and neoxanthin. The content of antheraxanthin in the light-grown cells of *Euglena gracilis* was much higher than that of β-carotene which is the main carotenoid in higher plants (Fig. 2).

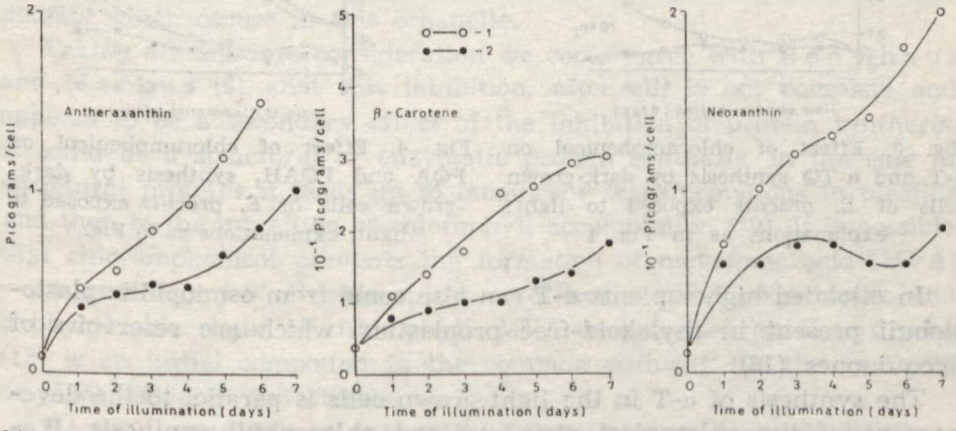

Fig. 2. Effect of chloramphenicol on antheraxanthin, β-carotene and neoxanthin synthesis by dark-grown resting cells of *E. gracilis* exposed to light; explanations as in Fig. 1

The cells of *Euglena gracilis* exposed to light produced about five times more antheraxanthin than β-carotene and neoxanthin. A very low level of carotenoids was observed in the etiolated cells. The accumulation of carotenoid was connected with the light-induced plastid development. The inhibition of *Euglena* carotenoid formation induced by chloramphenicol was about 50%. It is generally assumed that carotenoid and chlorophyll pigments are interrelated (33). The two pigment systems are morphologically associated in the cell where they attach to the same or very similar proteins in grana or lamellae of chloroplast. This close association suggests the possibility of biosynthetic relationships, especially in the case of chloramphenicol inhibition.

α-T occurs in etiolated cells and in light-grown cells of *Euglena gracilis* (Fig. 3). The presence of α-T in the etiolated cells also stated by Baszyński et al. (4, 5) can be a result of α-T association with structures different than chloroplasts (32).

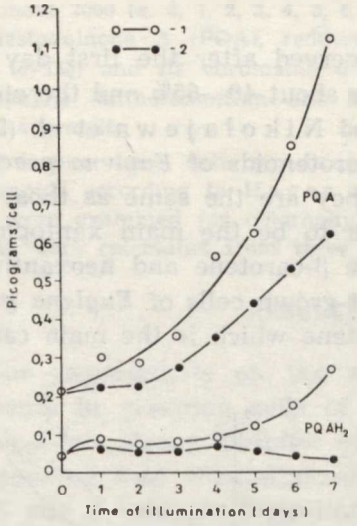

Fig. 3. Effect of chloramphenicol on α-T and α-TQ synthesis by dark-grown cells of *E. gracilis* exposed to light; explanations as in Fig. 1

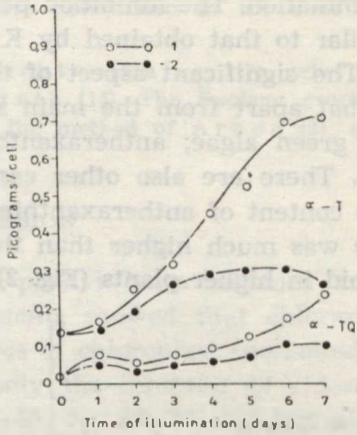

Fig. 4. Effect of chloramphenicol on PQA and $PQAH_2$ synthesis by dark-grown cells of *E. gracilis* exposed to light; explanations as in Fig. 1

In etiolated higher plants α-T can also come from osmiophilic plastoglobuli present in thylakoid-free proplastids, which are reservoirs of lipoquinones (16).

The synthesis of α-T in the light-grown cells is parallel to the development of the chloroplast structure and chlorophyll synthesis. Baszyński et al. (4, 5) showed that the well developed fine structure of chloroplast is necessary for the synthesis of the main quantity of

α-T. The content of α-T in chloramphenicol-treated cells was considerably lower than in those in the light and showed a slight decrease after seven days of illumination.

There was also observed an increase of α-TQ, PQA and $PQAH_2$ amount in the course of illumination of etiolated non-dividing *Euglena* cells and the inhibition of this process by chloramphenicol (Fig. 3—4). These compounds are very important participants in the electron transport of the photochemical processes in photosynthesis. The particularly rapid increase of PQA amount is an evidence for the photosynthetic activity of the cells.

The synthesis of these benzoquinones in greening cells of *Euglena gracilis* in the presence or absence of chloramphenicol is similar or parallel to chlorophyll synthesis and to chloroplast development. The content of benzoquinones in the chloramphenicol-treated cells was considerably lower when compared with that in the light-grown cells. The reduction of benzoquinone synthesis in the chloramphenicol-treated cells in relation to the control seems to indicate that light does not induce this synthesis if the cell is deprived of mature chloroplasts.

Gassman and Bogorad (11) thinks that chlorophyll formation is prevented by inhibitors of protein synthesis. They suggest that one or more enzymes involed in δ-aminolaevulinate (ALA) formation are labile, and require continuous synthesis; when such synthesis is prevented the enzyme level rapidly falls and thus chlorophyll formation comes to a stop. The constant ratio of benzoquinones to chlorophyll shows that chloramphenicol inhibits plastid benzoquinone synthesis to the same extent as pigment synthesis. It is an evidence for the correlation between the chloroplast structure and synthesis of the compounds which occurs in this organelle.

Taking all this into consideration we could agree with Ben Shaul and Markus (6), that this inhibition, after all, is not complete and appears to be a secondary effect of the inhibition of protein synthesis. It could be a structural or enzymatic protein synthesis. In the case of structural proteins it leads up to membrane transformations in plastids and then to the inhibition of chlorophyll accumulation (29). It is possible that chloramphenicol prevents the formation of mevalonic acid (MVA) — a precursor of all chloroplast carotenoids and lipoquinones, and a phytyl side chain of chlorophyll. MVA as proposed by Goodwin (13) is an initial compound in the common pathway for the synthesis of plant terpenes and sterols.

Thorne and Kodicek (31) in their investigations on the synthesis of the unsaponifiable lipids of *Lactobacillus casei* from mevalonic acid have shown that chloramphenicol inhibits the uptake of MVA.

Protein synthesis is necessary for MVA incorporation. The synthesis of proteins and synthesis of the lipids from MVA are closely connected, possibly for the simultaneous formation of the same lipoprotein structure i.e. of chloroplast.

Summing up it can be concluded that: 1) chloramphenicol inhibits the synthesis of plastid benzoquinones, similarly as pigments, in greening cells of *Euglena gracilis;*

2) the inhibition of plastid benzoquinone and pigment synthesis is connected with the inhibition of chloroplast protein formaton by chloramphenicol.

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STRESZCZENIE

Badano wpływ chloramfenikolu na syntezę benzochinonów i barwników plastydowych w spoczynkowych komórkach *Euglena gracilis*, inkubowanych na świetle. W zebranych w określonych odstępach czasu kulturach *Euglena gracilis* oznaczano benzochinony metodą Lichtenthalera (17), natomiast barwniki karotenoidowe metodą Hagera i Bertenratha (14). Zawartość chlorofilu oznaczono według Arnona (3).

Stwierdzono, że chloramfenikol hamuje syntezę PQA, $PQAH_2$, α-TQ-, α-T oraz chlorofilu, β-karotenu, anteraksantyny i neoksantyny w procesie zielenienia etiolowanych komórek *Euglena gracilis*. Zjawisko to jest prawdopodobnie wynikiem wpływu chloramfenikolu na syntezę strukturalnych i enzymatycznych białek chloroplastowych i jest wyrazem zależności między strukturą chloroplastu a syntezą składników obecnych w tych organellach.

РЕЗЮМЕ

Исследовалось влияние хлорамфеникола на синтез бензохинонов и пластидных красителей в покоящихся клетках *Euglena gracilis* инкубированных на свету. В собираемых через определенные промежутки времени культурах *Euglena gracilis*, бензохинон определялся методом Лихтенталера (17), а каротиноидные красители — методом Хагера и Бертенрата (14). Содержание хлорофила определялось по методу Арнона (3).

Установлено, что хлорамфеникол тормозит синтез PQA, $PQAH_2$, α-TQ-, α-T и хлорофила, β-каротена, антераксантина и неоксантина в процессе зеленения этиолированных клеток *Euglena gracilis*. Это явление, по всей вероятности, является следствием влияния хлорамфеникола на синтез структуральных и энзиматических хлоропластовых белков. Кроме того, это явление выражает зависимость между структурой хлоропласта и синтезом компонентов, содержащихся в этих органеллах.