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THE EFFECT OF CYCLOHEXIMIDE ON THE FINE STRUCTURE OF BEAN CHLOROPLASTS

Mit deutscher und kroatischer Zusammenfassung

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Introduction

Cycloheximide is a very effective inhibitor of protein synthesis. It acts on the level of cytoplasmic ribosomes (80s), and apparently does not affect chloroplast (70s) ribosomes (Ellis 1969). Since it is believed that some of the structural proteins of the chloroplast thylakoids are synthesized by cytoplasmic ribosomes (Kirk 1970), it seemed to be of interest to study the effect of cycloheximide on the fine structure of chloroplasts in higher plants.

Material and Methods

Primary bean leaves (*Phaseolus vulgaris*, cv. »Butterfisole«) at various stages of development were treated with 0.001%, 0.01% or 0.1% solutions of cycloheximide in tap water. The surface of the leaves was either wetted with the solutions in intervals of two hours, or the detached leaves were spread on the filter paper soaked with the same solution for 6, 30 or 48 hours. The material was kept under artificial discontinuous white light (mercury bulb VTF — 250 W TEŽ, illuminating intensity 5000 lx; 15 hours of light and 9 hours of darkness daily). Whole plants were also treated, usually by immersing the root in a 0.0001% or 0.001% solution of cycloheximide for 6 or 15 hours.

Two days after the beginning of the experiment small pieces of treated and control leaves were fixed in 1% glutaraldehyde, postfixed in OsO_4 and embedded in araldite. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined in a Siemens Elmiskop I.

Chlorophyll was extracted with 80% ethanol and the optical densities were measured with a Spekol (Carl Zeiss — Jena) spectrocolorimeter at the wavelength of 665 nm (Holden 1965). The concentrations of the chlorophyll were expressed in percentages as relative values in regard to the control material.

Results

When whole plants are treated with cycloheximide considerable damages appear in the fine structure of the leaf cells. The chloroplasts are remarkably altered. They become very small and the number of grana thylakoids is considerably reduced in comparison with the control. The plastid stroma is almost empty. Besides chloroplasts, other cell organelles are also damaged, especially the mitochondria, which are empty and swollen. The grade of the damage of the leaf cells is proportional to the concentration of the inhibitor and to the length of time of the treatment. In extreme cases the treatments are lethal causing the plants to die after a few days.

Much more specific changes in the fine structure of the chloroplasts are produced, if only the leaves are brought in contact with the solution. The strongest effect can be achieved on etiolated or green young, i.e. not fully developed, leaves.

In *etiolated leaves* the thylakoid system remains rudimentary and the remainders of prolamellar bodies exist even two days after the beginning of illumination regimen (Fig. 2).

If *young green leaves* are used for the experiment, the chloroplasts become roundish and much smaller than in the control. Their thylakoid system is curiously changed as well. Instead of straight and more or less parallelly arranged grana of the control (Fig. 1), only scarce grana are present, which are lying in the plastid stroma in various directions. Their diameter is somewhat larger, they are bent (Figs. 3, 4, 5), and are often in connection with one or more "tubular complexes" (Newcomb 1967; Figs. 5, 6). Sometimes, vacuoles appear in the plastids (Fig. 5). Depending on the concentration of cycloheximide used in such leaves the concentration of the chlorophyll is lowered to about 70—80% in comparison to the control. The treated leaves remain green, but the leaf elongation is practically stopped.

The changes described in the fine structure of the chloroplasts, namely the bending of the grana, happen only in young, not fully developed leaves. If *fully developed leaves* are treated, the thylakoid system remains unchanged except that in certain places the interthylakoid space fuses with the neighbouring membranes into a thick electron dense layer (Fig. 7).

The plastid stroma remains normal, and its ribosomes are well preserved after the treatment with low concentrations of cycloheximide. After treatment with high concentrations of the inhibitor (0.1%), however, crystalloids, which are especially numerous in fully grown leaves, appear in the stroma (Fig. 7). The crystalloids are built up of dark globular particles (of about 5 nm diameter) which are — in sections — arranged in rows of straight or somewhat bent lines 10 nm apart. Sometimes, another line of symmetry also appears, which is more or less prependicu-

lar to the first. It should be pointed out that such a treatment is lethal, so that the leaves die a few days later.

The fine structure of other cell organelles (e.g. mitochondria) remains almost normal for several days. The lumina of the endoplasmic reticulum and of the nuclear membrane are somewhat dilated (Fig. 3), and large osmiophilic globules appear in the cytoplasm (Figs. 3, 5); this is the first symptom of cell necrobiosis.

Discussion

The effect of cycloheximide on the fine structure of leaf cells — when whole plants are treated — does not seem to be a specific one, and is probably the consequence of some disorder in the normal metabolism of the cells. Cycloheximide is known to produce disturbances in ion uptake and energy transport of non-green plant tissue (MacDonald and Ellis 1969).

If only leaf cells are in contact with the solution, the changes seem to be more specific, both in developing etiolated, and in green leaves. The complete surface of the thylakoid system in the chloroplasts is remarkably reduced. In such leaves the chlorophyll concentration is considerably lowered as well. It is known that chlorophyll synthesis is inhibited by cycloheximide, but — according to the present knowledge — only indirectly by inhibiting the synthesis of structural proteins of the thylakoids (Kirk and Allen 1965, Kirk 1968, Hoober and Siekevitz 1968, Ben-Shaul and Ophir 1970). Cycloheximide inhibits the transfer of amino-acyl sRNA to the ribosomes, and subsequent protein formation in yeast cells (Siegel and Sisler 1964, 1965) and it affects the cytoplasmic ribosomes — at least in some higher plants (Ellis and MacDonald 1967, Jachymczyk and Cherry 1968, Davies and Maclachlan 1969) — but not the activity of chloroplast ribosomes (Ellis 1969).

The results obtained in this study seem to confirm the opinion that at least some of the precursors necessary for the thylakoid development are synthesized on the cytoplasmic ribosomes (Kirk 1970), although the effect of disturbances of other physiological processes should not be disregarded, as pointed out by MacDonald and Ellis (1969).

Groups of crystalloids, which can be seen in plastid stroma after severe damage of the cells with cycloheximide, seem to be identical with protein crystalloids observed in bean leaf chloroplasts after water loss (Wrischer 1967, 1970, Gunning, Steer and Cochrane 1968). According to Gunning et al. such inclusions represent the enzyme carboxydismutase, which forms crystalloids in been leaves very easily. A reliable interpretation of this phenomenon seems difficult, because the formation of such crystalloids can be caused by a variety of mechanisms (Wrischer 1970), e.g. after treatment of the leaves with simazine (Vrhovec 1971).

Osmiophilic globules, which appear in the cytoplasm of leaf cells after cycloheximide treatment, are probably signs of the so-called lipophanerosis, occuring during the cell necrobiosis (Wrischer 1965). Such inclusions have been described by other authors in the cytoplasm of plant cells after cycloheximide treatments (De Vecchi 1971).

Summary

Changes in the fine structure of bean chloroplasts after cycloheximide treatments are described. In greening etioplasts, and in young, not fully developed chloroplasts, cycloheximide causes considerable changes in the thylakoid system. Grana, in so far developed, are enlarged and bent; tubular complexes often appear. In fully developed chloroplasts some interthylakoid spaces appear as dark layers. After high concentrations of cycloheximide (0.1%) characteristic crystalloids appear in the plastid stroma.

Fig. 1. Part of a chloroplast from a young, untreated leaf. 36,000 : 1.

Sl. 1. Dio kloroplasta iz mladog, netretiranog lista. 36 000 : 1.

Fig. 2. Plastid from an etiolated leaf treated for 30 hours with a 0.01% solution of cycloheximide. Remainders of the prolamellar body and poorly developed thylakoids. 40,000 : 1.

Sl. 2. Plastid iz etioliranog lista tretiranog 30 sati 0,01% otopinom cikloheksimida. Ostaci prolamelarnog tjelešca i slabo razvijeni tilakoidi. 40 000 : 1.

Fig. 3. Cells from a leaf treated for 48 hours with a 0.1% solution of cycloheximide. Spherical chloroplasts contain bent grana. Dilated endoplasmic reticulum and nuclear membrane (arrows), osmiophilic globules in the cytoplasm *(o)*. 9,200 : 1.

Sl. 3. Stanice lista tretiranog 48 sati 0,1% otopinom cikloheksimida. Sferični kloroplasti sadrže svinuta grana. Endoplazmatski retikulum i jezgrina membrana dilatirani (strelice); osmiofilni globuli u citoplazmi *(o)*. 9 200 : 1.

Fig. 4. Chloroplasts from a leaf treated for 30 hours with a 0.01% solution of cycloheximide. Bent grana lying in all directions. 24,000 : 1.

Sl. 4. Kloroplasti iz lista tretiranog 30 sati 0,01% otopinom cikloheksimida. Svinuta grana leže u svim smjerovima. 24 000 : 1.

Fig. 5. Chloroplast from a leaf treated for 48 hours with a 0.1% solution of cycloheximide. Bent grana, tubular complex *(t)* and vacuoles in the stroma. Osmiophilic globules *(o)* in the cytoplasm. 24,000 : 1.

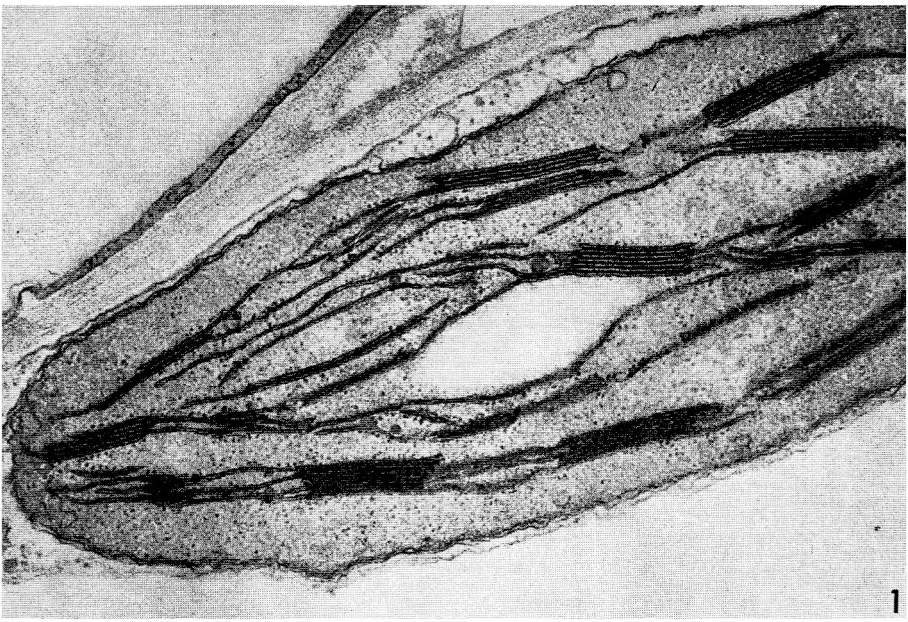
Sl. 5. Kloroplast iz lista tretiranog 48 sati 0,1% otopinom cikloheksimida. Svinuta grana, tubularni kompleks *(t)* i vakuole u stromi. Osmiofilni globuli *(o)* u citoplazmi. 24 000 : 1.

Fig. 6. Part of a chloroplast from a leaf treated for 48 hours with a 0,1% solution of cycloheximide. Bent grana in connection with a tubular complex. 50,000 : 1.

Sl. 6. Dio kloroplasta iz lista tretiranog 48 sati 0,1% otopinom cikloheksimida. Svinuta grana u vezi s tubularnim kompleksom. 50 000 : 1.

Fig. 7. Part of a chloroplast from a fully grown leaf treated for 48 hours with a 0.1% solution of cycloheximide. Dark crystalloids in the stroma *(c)*. Thickenings of thylakoid membranes (arrow). 80,000 : 1.

Sl. 7. Dio kloroplasta izraslog lista tretiranog 48 sati 0,1% otopinom cikloheksimida. U stromi tamni kristaloidi *(c)*. Zadebljanja tilakoidnih membrana (strelica). 80 000 : 1.



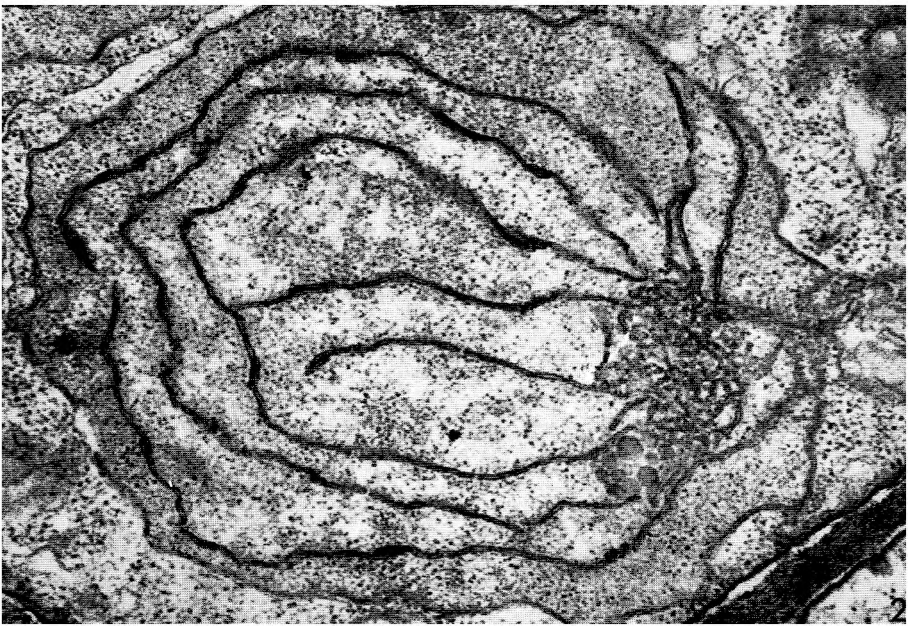


Fig. 1—2.

Fig. 3.

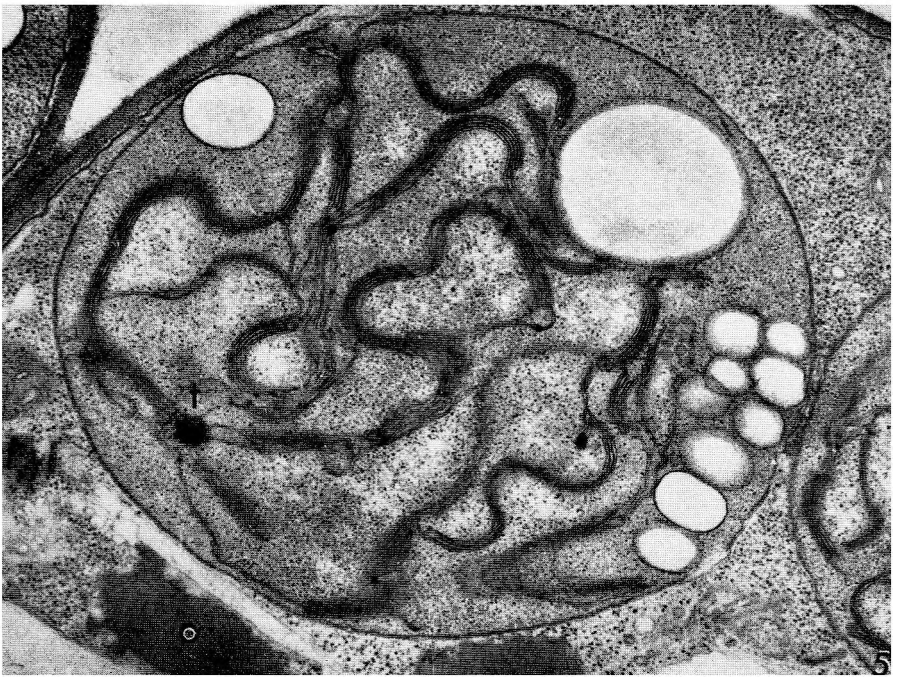


Fig. 4—5.

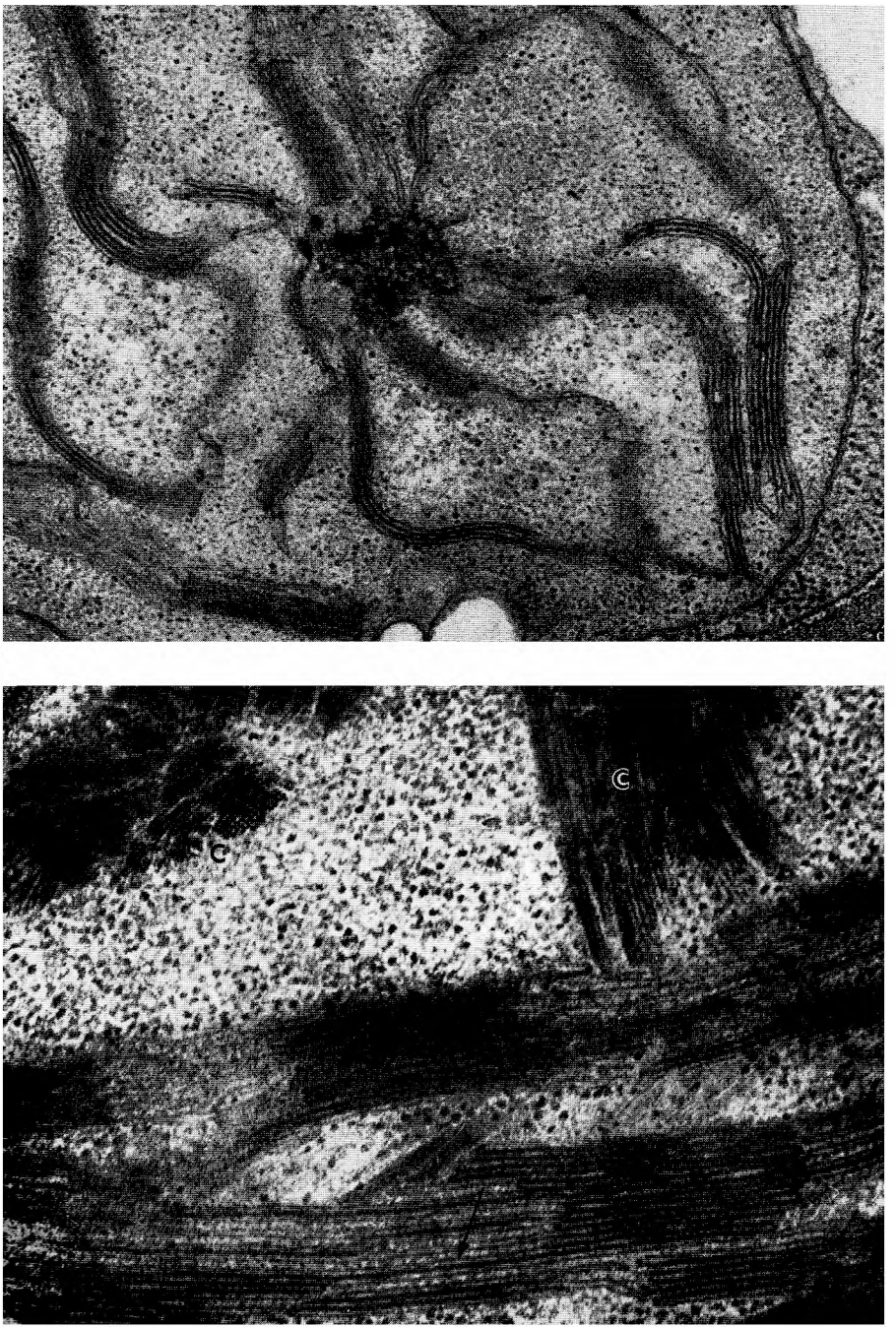


Fig. 6—7.

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ZUSAMMENFASSUNG

DIE WIRKUNG VON CYCLOHEXIMID AUF DIE FEINSTRUKTUR DER BOHNEN-CHLOROPLASTEN

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Veränderungen in der Feinstruktur der Bohnen-Chloroplasten nach Behandlung mit Cycloheximid werden beschrieben. In ergrünenden Etioplasten, sowie in jungen, noch nicht völlig entwickelten Chloroplasten, verursacht Cycloheximid auffallende Veränderungen im Thylakoid-System. Grana-Thylakoide, sofern vorhanden, sind vergrössert und gebogen; häufig kommen tubuläre Komplexe vor. In voll entwickelten Chloroplasten erscheinen einzelne interthylakoidale Räume als dunkle Schichten. Nach Behandlung mit hohen Konzentrationen von Cycloheximid (0.1%) entstehen in Chloroplasten-Stroma charakteristische Kristalloide.

SADRŽAJ

UTJECAJ CIKLOHEKSIMIDA NA FINU GRAĐU KLOROPLASTA GRAHA

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Opisane su promjene u finoj građi kloroplasta graha nakon djelovanja cikloheksimida. U etioplastima, koji se nalaze u fazi ozelenjavanja, kao i u mladim, još nepotpuno razvijenim kloroplastima, cikloheksimid uzrokuje znatne promjene u tilakoidnom sustavu. Grana, ukoliko ona postoje, povećana su i svinuta, a pojavljuju se često i tubularni kompleksi. U razvijenim kloroplastima pojedini intertilakoidni prostori stvaraju zajedno s membranama tamni sloj. Nakon visokih koncentracija cikloheksimida (0,1%) u stromi kloroplasta pojavljuju se karakteristični kristaloidi.

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