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THE EFFECT OF 1-O-(INDOL-3'-YLACETYL) β-D-GLUCOPYRANOSE AND INDOL-3-YL-ACETIC ACID ON SOME UNICELLULAR GREEN ALGAE

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Introduction

The IAA-glucose ester 1-O-(indol-3'-ylacetyl)-ß-D-glucopyranose (IAβG) synthesized by Keglević and Pokorny (1969) in the Institute "Ruđer Bošković" shows a higher activity in Avena coleoptile elongation tests as compared with the action of exogenous IAA itself. In order to investigate the biological activity of the new compound on different biological systems a study of the effect on growth of some unicellular green algae has been attempted.

The unfavourable circumstances in investigating the effect of auxins on algae is the great variability in responses depending on species and even strains of the algae used (see reviews by Thimann and Beth 1959, Lang 1965 and by Ahmad and Winter 1968). The stimulating effects of auxins on algae have been investigated as an increase in the cell number, in dry weight, in the elongation of individual cells and, in multicellular algae, as changes in the organ formation. The surprising finding of Ahmad and Winter (1968) is the stimulation of growth at very high concentrations of IAA (10^{-3} M) of the green unicellular algae tested and no inhibition of growth.

Material and Methods

In the present study the green unicellular algae: *Chlamydomonas reinhardii* Dangeard, *Scenedesmus quadricauda* (Turpin) Brébisson, *Euglena gracilis* Klebs strain »Z« and *Chlorella vulgaris* s. l. were cultured in the inorganic medium (for *Euglena* vitamins B_1 and B_{12} were added) of Sager and Granick (1954). The cultures were illuminated for 14 hours daily with fluorescent lamps providing the algae with a light intensity of 150 ± 10 ft-c. *Euglena* was kindly supplied by Dr. Birnboim (Chalk River Nuclear Laboratories, Canada), *Chlamydomonas* from the Cambridge Culture Collection, while *Scenedesmus* and *Chlorella* were isolated by the author. The stock cultures of axenic algal strains were kept on rich organic medium of Gibbs (1960) with beef extract omitted and supplied with 0.1 per cent glucose. The use of alcohol as solvent for IAA was avoided (IAßG is easily soluble in water).

Aliquots of algae (ca 10^4 cells/ml) were inoculated in 10 ml medium in the test tubes. The cell number after 5 days of growth was measured in a haematocytometer.

In viability tests, after growth in the medium + IAA or IAßG the cells were washed several times with fresh medium and plated on rich organic agar medium. After 7 days the plates were examined for colony formation.

Results and Discussion

At low concentrations of IAA or IAβG (from 0.01 to 1.0 μM) there has been no marked stimulation or inhibition of growth (as expressed in cell number) in all the unicellular algae tested. As *Chlamydomonas reinhardii* shows an inhibition of growth at high concentrations of auxins, it was tested for their inhibitory action. At the concentration of 10^{-3} M IAA, which is stimulatory to growth of some unicellular algae (Ahmad and Winter 1968), there has been a complete inhibition of growth in *Chlamydomonas*, followed by the loss of flagella and subsequent death of the cells (Fig. 1). For comparison normal flagellated living cells are represented in Fig. 2.

In viability tests there has been no recovery of the IAA-treated cells.

At equimolar concentrations of IAβG the inhibition of growth is much less pronounced. The loss of flagella occurs in only about 30 per cent of cells, and the formation of 4-flagellated and giant cells appears as a consequence of the inhibition of cell division without growth inhibition (Fig. 3a and 3b). The comparison of growth of control cells and those grown in 10^{-3} M IAA and IAβG is shown in Fig. 4.

Fig. 1 Culture of *Chlamydomonas reinhardii* after 5 days of growth in 10^{-3}M IAA. Cells are dead. Anoptralcontrast, 600 : 1.

Fig. 2 *Ch. reinhardii*, after 5 days of growth, control. Anoptralcontrast, 600 : 1.

Fig. 3a *Ch. reinhardii*, after 5 days of growth in 10^{-3} M IAßG (part of the culture from the bottom of the tube). Note the 4-flagellated, giant cells as well as normal ones. Anoptralcontrast, 600 : 1.

Fig. 3b Giant cells of *Ch. reinhardii* induced by 10^{-3} M IAßG. Anoptralcontrast, 600 : 1.

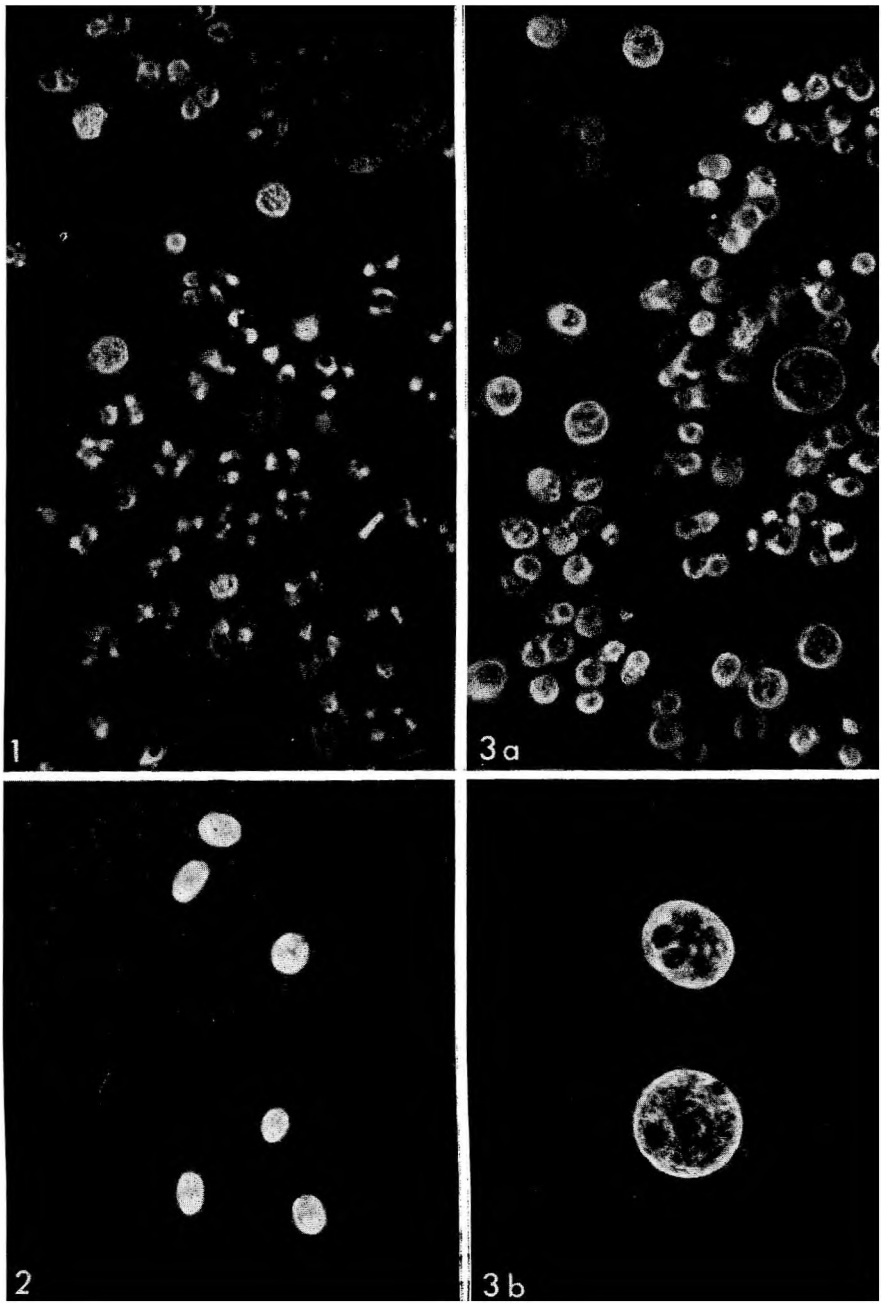

Fig. 1 — 3.

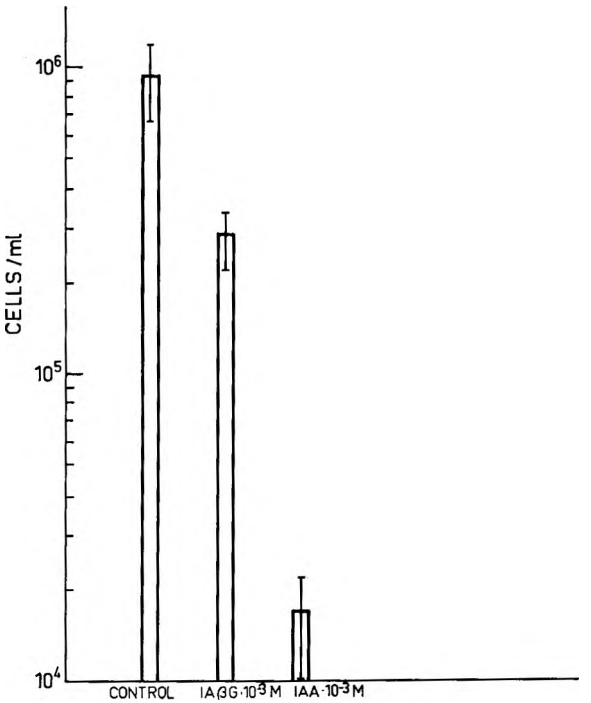

Fig. 4 Comparison of the inhibitory effect of equimolar concentrations of IAA and IAßG on growth of *Ch. reinhardii*. The columns represent the mean values of 3 experiments after 5 days of growth.

In viability tests there has been a recovery of IAβG-treated cells, except for the giant cells which are irreversibly changed. In equimolar concentrations of IAA only few giant cells were found. This increase in cell size of the individual cells (the formation of giant cells) should be regarded not as a specific action of auxins, but probably as a result of unbalanced growth, like that occuring after ionising irradiation or after treatment with drugs like bromodeoxyuridine, or in old cultures. Abnormally developed cells in high concentrations of IAA were observed also by Davidson (1952) in *Rhizoclonium*.

Thin layer chromatography of the IAβG-medium has revealed that the compound has not been decomposed by algae growing in the medium for 7 days. IAβG and only traces of IAA could be detected on the chromatogram.

Since in the blue-green algae a stimulatory effect on growth at very low doses of IAA has been observed (Bunt 1961, Ahmad and Winter 1968a, 1968b and 1969), it seems more rewarding to test the effects of IAβG on these organisms.

Summary

The results of the present study have thus shown that low concentrations (0.01—1.0 μM) of IAA and IAβG do not stimulate the division of the unicellular algae tested.

High concentrations of IAA and IAβG (10^{-3} M), tested only on *Chlamydomonas reinhardii*, inhibited the cell division with subsequent loss of flagella and giant cell formation.

The results indicate a lower toxicity of IAβG in comparison to the effect of IAA.

I am indebted to Dr. D. Keglević for the sample of 1-O-(Indol-3'-ylacetyl)-β-D-glucopyranose and to Dr. M. Pokorny for carrying out the thin layer chromatography.

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SADRŽAJ

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Niske koncentracije IAA i IAβG (0,01—1,0 μM) ne stimuliraju diobu ispitivanih jednostaničnih alga.

Visoke koncentracije IAA i IAβG (10^{-3} m), koje su ispitane samo na vrsti *Chlamydomonas reinhardii*, inhibiraju staničnu diobu uz gubitak bičeva i pojavu gigantskih stanica.

Rezultati ukazuju na slabiju toksičnost IAβG u usporedbi s učinkom IAA.

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