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The effect of 3-indolylacetic acid on the accumulation of starch in the root tissue of *Cichorium intybus* L. cultured *in vitro*

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Abstract

The relation between IAA-induced formation of amyloplasts in callus cells of chicory root and the influence of IAA on sugar uptake from the medium was investigated. Experiments with ^{14}C-sucrose showed that IAA increased the uptake of sucrose from the medium. The amyloplast-like structures were also observed in callus grown on medium without IAA, but containing high concentration of sucrose (9%). The possibility of IAA influence on the formation of amyloplasts by increasing the permeability of cells for sugar is discussed.

INTRODUCTION

In a previous paper it has been reported that in callus of chicory root explants cultured at relatively low sugar level, 3-indolylacetic acid (IAA) induces development of proplastids into amyloplasts, while in the absence of IAA chloroplasts are formed (Woźny et al. 1973).

In the present study, the effect of auxin on sugar uptake and the relation between polysaccharide formation and sugar supply of the tissue have been investigated.

MATERIAL AND METHODS

Roots of *Cichorium intybus* L. var. *sativum* cv. Polanowicka were surface sterilized with 0.2% $HgCl_2$ and washed with sterile water. Upper (basal) root parts were sectioned into disks of 1 cm thickness from which cylinders 0.6 cm in diameter were cut out, composed of

phloem, cambium and xylem. They were planted (with their basal, shoot directed ends turned down) in culture tubes on Murashige and Skoog mineral solution, solidified with 0.8% agar, and containing sucrose and IAA at various concentrations. The explants were grown at 22±2°C, at a humidity of about 70% and in continuous white fluorescent light of about 800 lux.

For electron miscroscopy observations small fragments of callus tissue (ca. 1 mm^3) were taken after 7 days of culture, fixed in 3% glutaraldehyde in 0.1 M phosphate buffer of pH 7.5 and postfixed in 2% OsO_4 in the same buffer. The material was dehydrated in ethanol series and propylene oxide, embedded in Epon 812 and sectioned by LKB ultramicrotome. Photographs were taken on the JEM 7A electron microscope.

For investigations with ^{14}C-sucrose, initial explants (directly excised from roots) and explants after 1, 3, 6 or 7 days of culture on medium containing 1.5% sucrose without IAA (control) and with IAA at 10 or 100 mg/l were used. Disks (6 mm in diameter and of 2 mm thickness) were cut out from explants (initial and 1-day old material) or from callus (3-, 6- and 7-day old material). Before incubation with ^{14}C-sucrose disks of initial explants were conditioned for 3 hours in 0.025M Tris-HCl buffer of pH 7.5 containing 1.5% sucrose without IAA (control) or with the addition of IAA at 10 or 100 mg/l. The incubation medium had the same composition as the conditioning solution, with the addition of ^{14}C-sucrose (UVVVR Prague-Czechoslovakia, spec. act. 0.1 mCi/mM) at a concentration of 0.05 μCi/ml. The incubation was carried out on a shaker for 2 hours. After that, tissue disks were washed in 0.025M Tris-HCl buffer of pH 7.5 and dried at 60°C to constant weight. The dry tissue was ground in a mortar, 4 mg samples of material were placed in toluene scintillator, and their radioactivity was measured in scintillation counter USB-2.

RESULTS AND DISCUSSION

In conditions of high rate of sugar synthesis in cell, the sugar is transformed in a physiologically and osmotically less active form, usually by condensation into starch. Studies of Moore and Lovell (1972) on excised cotyledon petioles of *Sinapis*, and in particular investigations of Kordan (1971b, 1972) on lemon fruits showed that supplying the plant organs with exogenous sugar leads also to accumulation of starch in tissues. Kordan observed extensive starch deposits also in non-proliferating explants (1971a) and in tissue cultures of lemon fruits (1963, 1969). On the basis of numerous experiments he concluded that formation and accumulation of starch in plant tissues

are possible as long as sucrose is accumulated in amounts that exceed the metabolic requirements of cells.

Considering this it seemed interesting to examine first if the formation of amyloplasts in chicory callus tissue is related to an increased sugar supply of the tissue. Turkina and Sokolova (1972) have found that the uptake of sucrose by cells of sugar beet is proportional to its concentration in the medium. Therefore, in a first series of experiments the effect of sucrose concentration on tissue growth and on the development of amyloplasts was investigated. It has been found, that sucrose stimulates growth of the callus with a maximum at a concentration of 9% (Fig. 1). With the increase of sucrose concentration in

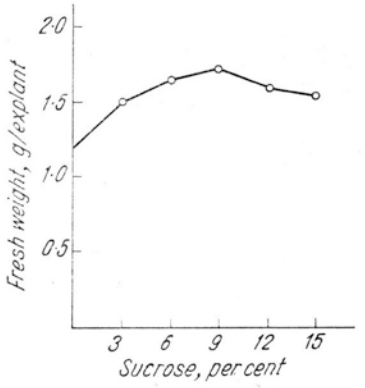

Fig. 1. Effect of sucrose concentration on the growth of explants after 14 days of culture

medium, the callus tissue became more compact, its colour changed from white to yellow-green, with a tendency to browning. Shoots developing on callus gradually decreased in number and size. The results were in agreement with those obtained by Gwóźdź and Szweykowska (1969a) on the same plant material.

Examination of ultrastructure of callus cells produced on low and high sugar level in the medium was carried out with explants grown for 7 days in the presence of sucrose at 1.5 and 9%, resp. After 7 days of culture the explants on control medium (1.5% sucrose) produced small amount of a light-green callus. On medium with 9% sucrose the callus was yellow-green, very firm and fragile. In both cases the electron microscope analysis revealed, that parenchymatic callus cells were relatively strongly vacuolised, with only a thin layer of cytoplasm. The plastids on 1.5% sucrose contained thylakoid systems, the compartments of which often showed swelling (Fig. 2). The plastids of callus cells grown on medium with 9% sucrose were filled with starch-like grains (Fig. 3) which, however, did not show characteristic blue staining with I_2/KI. They were probably polysaccharide grains of a character somewhat different from starch. This might be connected with the fact,

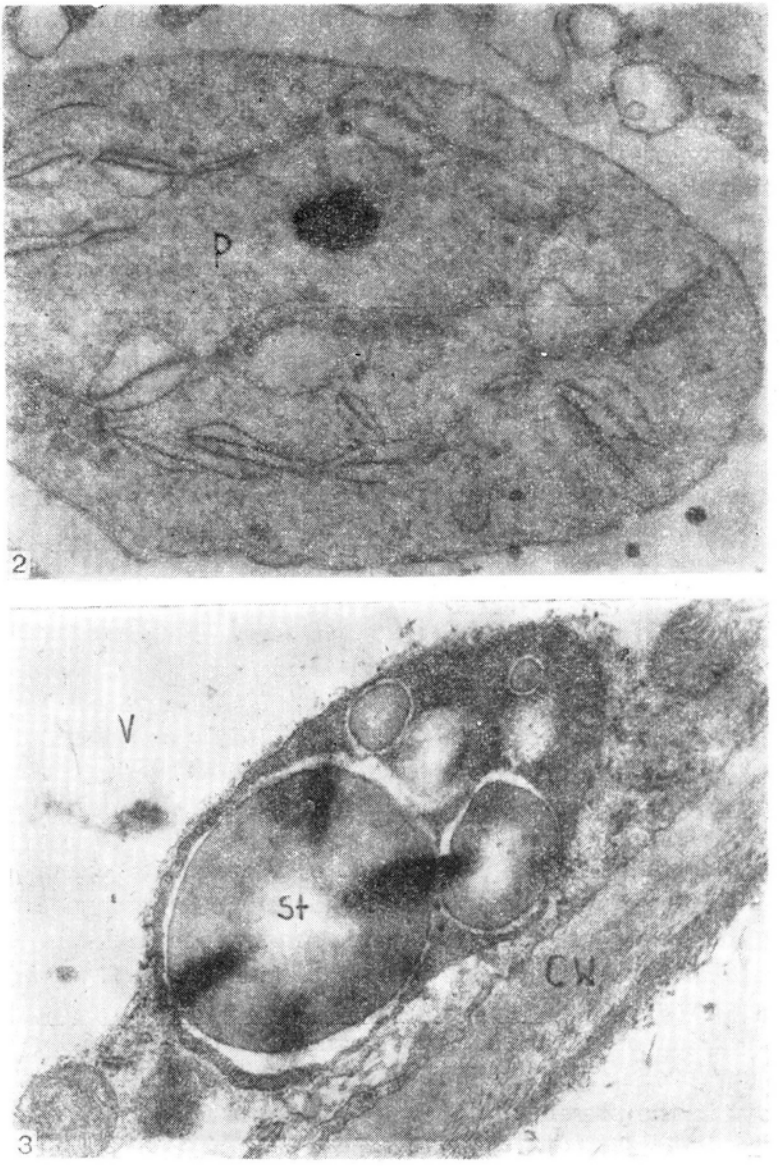

Fig. 2. Plastid from callus grown for 7 days on medium with 1.5% sucrose. Glutaraldehyde and OsO_4. × 28 000

Fig. 3. Amyloplast with starch-like grains from callus grown for 7 days on medium with 9% sucrose. Glutaraldehyde and OsO_4. × 33 800

that inulin is the normal storage material in the chicory root. It is also known that plant tissues in the *in vitro* culture may accumulate products different from those which are stored in plants in normal conditions (Krikorian and Steward 1969).

Woźny et al. (1973) observed formation of amyloplasts in the same plant material and in similar conditions of culture, on medium contain-

ing, besides 1.5% sucrose, IAA at 10 or 100 mg/l. Starch formation under the influence of another auxin, 2,4-D, was also observed by Sunderland and Wells (1968) in *Oxalis* callus culture. The stimulation by auxin of starch synthesis might be a result of changes in cell metabolism, but it also might simply result from an increased sugar uptake. In a second series of experiments, tissues were incubated with ^{14}C-sucrose and after 2 hour incubation their complete radioactivity was measured. The results are presented in Figs. 4 and 5. The initial mate-

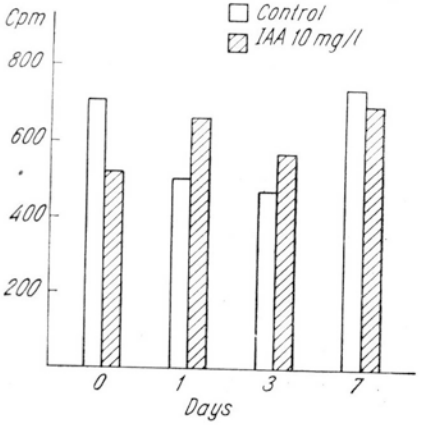

Fig. 4

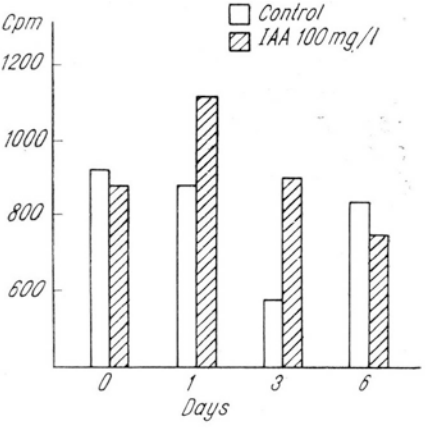

Fig. 5

Figs. 4 and 5. Effect of IAA on the uptake of ^{14}C-sucrose by initial (O time) explants from chicory root, by explants after 1 day of culture and by calluses from explants after 3 and 7 days of culture in medium with sucrose at 1.5% and IAA (no IAA in control medium). Tissue samples were incubated for 2 h in solution containing 0.025 M Tris-HCl, pH 7.5, sucrose at 1.5%, ^{14}C-sucrose at 0.05 μCi/ml and IAA (no IAA in the control solution)

rial (time 0) did not respond to the presence of auxin with an increased uptake of sucrose, on the contrary, the uptake was somewhat lower, especially at 10 mg/l of IAA. A similar inhibition of elongation of corn coleoptiles at an early time of auxin action was observed by Rayle et al. (1970). However, an increased uptake of sucrose by chicory tissue in relation to the control appeared after 1 and 3 days of IAA action.

It may be concluded that the auxin-induced starch formation in callus tissue developing on chicory root explants is more or less related to this increased sugar uptake. It is difficult to speculate on the way by which IAA affects sugar penetration into the tissue, however, it is known that growth substances affect the permeability of cells (v. Guttenberg and Beythien 1951; Masuda 1955, Humphreys and Dugger 1959, Pohl 1961). This explanation may be favoured by a previous finding of Gwóźdź and Szweykowska (1969b) that higher auxin concentrations also bring about an increased water content of chicory callus.

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Wpływ kwasu 3-indolilooctowego na akumulację skrobi w tkance korzeni Cichorium intybus L. hodowanej in vitro

Streszczenie

Badano zależność między tworzeniem się amyloplastów pod wpływem IAA w komórkach kalusa korzeni cykorii, a wpływem IAA na pobieranie cukru z pożywki. Doświadczenie z użyciem znakowanej sacharozy wykazało, że IAA zwiększa pobieranie sacharozy z pożywki. Amyloplasto-podobne struktury obserwowano również w kalusie rosnącym na pożywce bez IAA, lecz zawierającej wysokie stężenie sacharozy (9%). Autorki wyrażają przypuszczenie, że IAA może wpływać na tworzenie się amyloplastów poprzez zwiększenie przepuszczalności komórek dla cukru.