

Aspartate aminotransferase isoenzymes in cucumber roots as depending upon the mineral composition of the medium

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(Received: January 7, 1980)

Abstract

Aspartate aminotransferase (GOT) isoenzymes separated by means of electrophoresis on cellulose acetate gel were examined. Five GOT isoenzymes were discovered in cucumber roots after several days of their growth. The activity of these isoenzymes was dependent upon the mineral composition of the medium. If the medium did not contain nitrogen, isoenzymes 1, 3, and 4 showed only slight activity. Activity of all of the isoenzymes in the roots of seedlings assimilating NH_4^+ ions was higher than in the roots of seedlings assimilating NO_3^- ions. Higher activity was observed in all of the isoenzymes in the nitrate or ammonium medium lacking calcium, while only some showed higher activity at the lack of potassium.

INTRODUCTION

Aspartate aminotransferase (GOT) occurs in the cytoplasm as likewise in many cell organella (Splittstoesser and Steward 1970, Yamazaki and Tolbert 1970, Hatch and Mau 1973, Mifflin 1974). The enzyme contained in organella is located mainly in their soluble matrix, the membrane fraction shows but slight GOT activity (Wightman and Skinner 1978).

Liu and Huang (1977) observed that etiolated cotyledons of four days old cucumbers contain six GOT isoenzymes, of which two are glioxysomes and disappear after greening of plants, three are linked with proplastides, and one with the cytoplasm. Four various isoenzymes are present in spinach leaves, in which case the occurrence of each is limited to one subcellular fraction (Rehfeld and Tolbert 1972, Huang et al. 1976).

Previously obtained data (Kubik-Dobosz 1981a, 1981b) showed that GOT activity in cucumber seedlings depended upon the mineral

composition of the medium. In this connection it was decided to examine the influence of mineral nutrition on the level of activity of GOT isoenzymes in the roots of cucumbers growing in nitrate or ammonium medium differing with respect to the content of K^+ and Ca^{2+} ions.

MATERIAL AND METHODS

Cucumber (*Cucumis sativus* L. of the "Monastyrski" variety) seeds were germinated in distilled water at 27°C, in darkness. After two days the seedlings were transferred to media. The plants were given 17 h of light (5000 lux) and 7 h of darkness for 1 to 4 days. The following types of media were used: complete nitrate or ammonium (200 mg of N) medium, the composition of which has been presented previously (Kubik-Dobosz and Soroka 1979), medium lacking nitrogen, nitrate or ammonium medium not containing potassium or calcium ions. The cucumber roots were homogenized with an addition of chilled 0.04 M tris-HCl buffer (pH 7.5) containing 0.25 mM EDTA and 0.2% Triton X-100; 2.5 cm³ of the buffer was used per 1 g of roots. The homogenate was filtered through a Whatman 1 filter paper and the filtrate was used to electrophoresis. Electrophoresis was carried out on cellulose acetate gel. Strips of 40 × 170 mm from the cellulose acetate gel (Cellogel) were soaked for 10 minutes in a buffer solution (pH 7.2) containing: 6.35 mM Tris, 6.3 mM maleic acid, 0.64 mM EDTA, 0.62 mM MgCl₂. This buffer was also used to fill the electrophoresis apparatus according to Buczek and Dobosz (1976). Cellogel strips were lightly dried by means of filter paper and 3 mm³ of the enzymatic extracts deposited on each. Electrophoresis took place at a temperature of 4°C for 40 min. at 400 V (3 mA). Cellogel strips were next transferred to a staining base made up of 4 cm³ 2% agarose with 4 cm³ of a buffer contained 0.2 M tris-HCl (pH 7.8), 40 mM L-aspartate, 10 mM alfa-ketoglutarate, 0.2 mM pyridoxal phosphate, 50 units of glutamate dehydrogenase (GDH), 4 mg NAD⁺, 1 mg 3-(4,5-dimethyl-thiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT), 1 mg phenazine methosulphate (PMS). Staining took place in darkness at a temperature of 37°C for 30 minutes.

Chemicals. Tris (Hydroxymethyl) aminomethane was purchased from Koch-Light Laboratories Ltd. Enzyme cofactors, substrates and purified glutamate dehydrogenase were obtained from Sigma Chemical Co. Cellulose acetate gel (Cellogel) was produced by Chemetron, Italy.

RESULTS

After two days of germination of seeds, the roots showed a presence of five isoenzymes GOT of various activity (Fig. 1). The number of isoenzymes contained in the cucumber roots corresponded to the num-

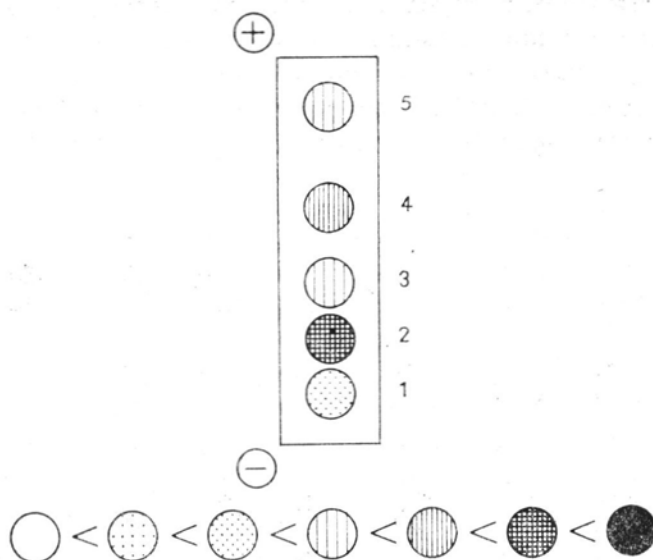


Fig. 1. Aspartate aminotransferase isoenzymes in cucumber roots after two days of germination in distilled water. Symbols order presents increasing enzyme activity

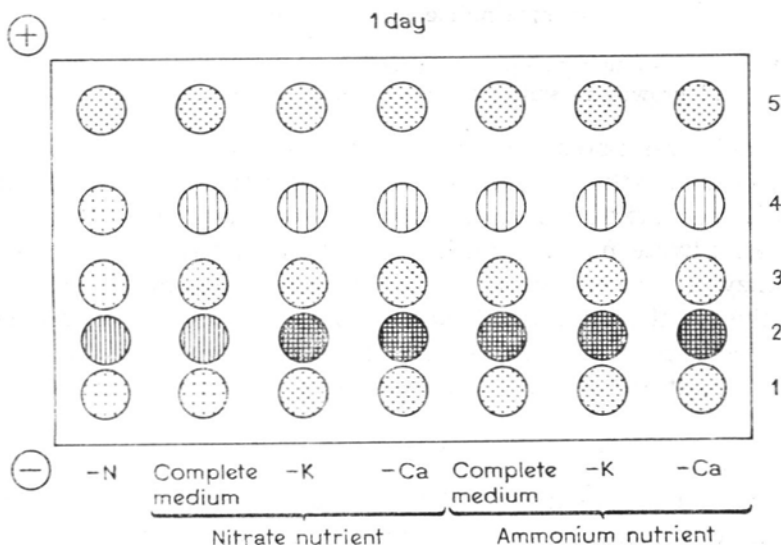


Fig. 2. Aspartate aminotransferase isoenzymes in cucumber roots after one day of growth in nutrient media. Explanations as in Fig. 1

ber obtained by Liu and Huang (1977) from etilated cotyledons of two day old cucumbers. After one day of growth of seedlings on nutrient media differing with respect to nitrogen content and potassium or calcium ions, it was noted that activity of certain isoenzymes depends

upon the presence of nitrogen in the nutrient media (Fig. 2). At further growth of the cucumber seedlings in differing media it was also noted that activity of particular isoenzymes differed in connection with the lack of K^+ or Ca^{2+} (Fig. 3). If the nutrient medium did not contain nitrogen, only trace activity of isoenzyme 1 and low activity of isoenzy-

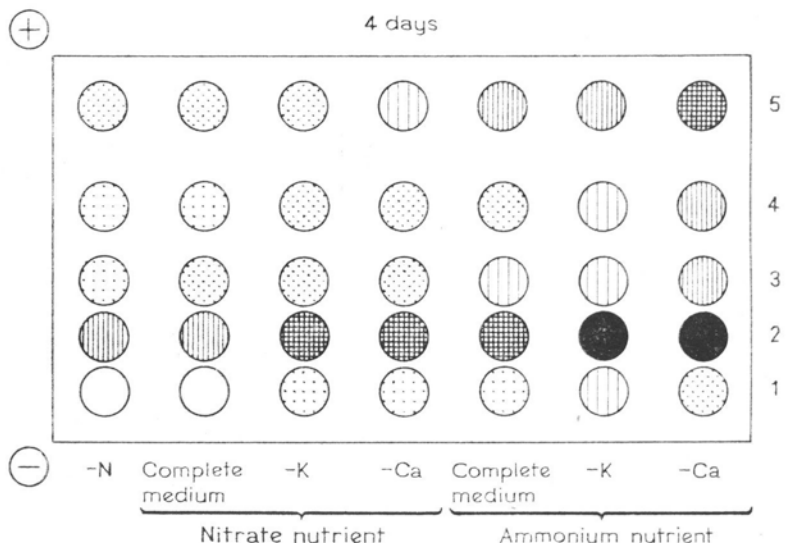


Fig. 3. Aspartate aminotransferase isoenzymes in cucumber roots after four days of growth in nutrient media. Explanations as in Fig. 1

mes 3 and 4 was noted after four days. On the other hand all of the isoenzymes in seedlings growing on a complete ammonium medium showed higher activity than isoenzymes from seedlings developing on a complete nitrate nutrient medium. Increased activity of almost all of the isoenzymes was noted with the lack of calcium in the medium, irrespective of the mineral nitrogen form used (Fig. 3). Increased activity of isoenzymes 1, 2 and 4 was noted after four days of growth of seedlings in nitrate or ammonium medium not containing K^+ ions.

DISCUSSION

Previous studies (Kubik-Dobosz 1981a, 1981b) showed increase GOT activity in the roots of cucumbers growing in the presence of ammonium ions, or at lowered availability of K^+ or Ca^{2+} ions. The present studies showed distinct correlation between increased GOT activity in the roots of cucumbers observed previously, and activity of GOT isoenzymes in the mentioned organs. It was namely found that higher GOT activity connected with the lack of K^+ in the medium is caused by the increase in the activity of certain isoenzymes (Fig. 3). Results obtained indicate that with respect to various GOT isoenzymes the

effect of the lack of K^+ in the medium is not uniform, this probably being linked with the location of particular isoenzymes in the various subcellular structures. Effect of NH_4^+ and Ca^{2+} ions has a more general character and is linked with increased activity of practically all of the isoenzymes. Studies of Epstein (1965) and Nilshammar et al. (1972) point to the role of calcium in the organization of cytoplasm and permeability of cytoplasmatic membranes. Lack of calcium causes disorganization of plasmatic membranes, changes their properties and in effect such membranes lose their selectivity. The participation of calcium ions in regulating activity of all GOT isoenzymes might be the result of disruption in the compartmentation of compounds regulating activity of this enzyme.

During the first days of cucumber seedlings development no increased activity of GOT isoenzymes was noted in the roots in the presence of nitrate ions, this probably being due to the considerably lower reduction of nitrates in the cucumber roots (Buczek and Burzyński 1979). Lower availability of the substrate (NH_4^+) for glutamate synthesis probably limits activity of the GOT isoenzymes. Ammonium ions assimilated in the roots from the external solution are rapidly included into the amino acid biosynthesis system (Muhammad and Kumazawa 1974), indirectly also influencing activity of GOT isoenzymes.

The results indicate that supplying of NH_4^+ ions to cucumber seedlings leads to a distinct increase of the activity of five GOT isoenzymes in the roots of these plants. A similar correlation was not observed if the plants assimilated NO_3^- exclusively. Lack of calcium in the nutrient media led to a significant increase in the activity of all of the GOT isoenzymes and the lack of K^+ to increased activity of only some. Results indicate that Ca^{2+} and K^+ ions regulate the level of activity of GOT isoenzymes.

Acknowledgments

This study was financed from Problem MR II/7.

REFERENCES

- Buczek A., Dobosz T., 1976. Aparat do elektroforezy na octanie celulozy. *Problemy Kryminalistyki* 119: 82-84.
- Buczek J., Burzyński M., 1979. Nitrate reductase activity and yield of dry mass and protein content in cucumber seedlings supplied with nitrates and ammonium. *Acta Soc. Bot. Pol.* 48: 465-471.
- Epstein E., 1965. Mineral Metabolism. In: *Plant Biochemistry*. Ed. Bonner J., Varner J. E., Acad. Press, pp. 438-466.

- Hatch M. D., Mau S. L., 1973. Activity, location, and role of aspartate aminotransferase and alanine aminotransferase isoenzymes in leaves with C_4 pathway photosynthesis. *Arch. Biochem. Biophys.* 156: 195-206.
- Huang A. H. C., Liu K. D. F., Youle R. J., 1976. Organelle-specific isozymes of aspartate- α -ketoglutarate transaminase in spinach leaves. *Plant Physiol.* 58: 110-113.
- Kubik-Dobosz G., 1981a. The effect of ammonium ions on the activity of glutamate dehydrogenase, alanine aminotransferase and aspartate aminotransferase in *Cucumis sativus* L. seedlings. *Acta. Soc. Bot. Pol.* 50: 429-436.
- Kubik-Dobosz G., 1981b. The influence of potassium and calcium ions on nitrogen metabolism of cucumber seedlings. *Acta Soc. Bot. Pol.* 50: 437-447.
- Kubik-Dobosz G., Soroka K., 1979. Influence of light and mineral nitrogen forms on the activity of some enzymes in *Cucumis sativus* L. cotyledons. *Acta Soc. Bot. Pol.* 48: 443-452.
- Liu K. D. F., Huang A. H. C., 1977. Subcellular localization and development changes of aspartate- α -ketoglutarate transaminase isozymes in the cotyledons of cucumber seedlings. *Plant Physiol.* 59: 777-782.
- Mifflin B. T., 1974. The location of nitrite reductase and other enzymes related to amino acid biosynthesis in the plastids of root and leaves. *Plant Physiol.* 54: 550-555.
- Muhammad S., Kumazawa K., 1974. Assimilation and transport of nitrogen in rice. I. ^{15}N -labelled ammonium nitrogen. *Plant Cell Physiol.* 15: 747-758.
- Nilshammar M., Waller B., Kylin A., 1972. The effect of calcium deficiency on the ultrastructure of the green alga *Scenedesmus*. *Z. Pflanzenphysiol.* 66: 193-196.
- Rehfeld D. W., Tolbert N. E., 1972. Aminotransferases in peroxisomes from spinach leaves. *J. Biol. Chem.* 247: 4803-4811.
- Splittstoesser W. E., Stewart S. A., 1970. Distribution and isoenzymes of aspartate aminotransferase in cotyledons of germinating pumpkins. *Physiol. Plant.* 23: 1119-1126.
- Wightman F., Skinner D., 1978. Aminotransferase activities of subcellular fractions from castor bean endosperm tissue. *Plant Physiol. (Suppl.)* 61: 67.
- Yamazaki R. Y., Tolbert N. E., 1970. Enzymatic characterization of leaf peroxisomes. *J. Biol. Chem.* 245: 5137-5144.

Izoenzymy aminotransferazy asparaginianowej w korzeniach ogórków w zależności od mineralnego składu pożywki

Streszczenie

Badano izoenzymy aminotransferazy asparaginianowej (GOT) rozdzielane metodą elektroforezy na octanie celulozy. W kilkudniowych korzeniach ogórków wykryto pięć izoenzymów GOT, których aktywność była uzależniona od mineralnego składu pożywki. Jeżeli pożywka nie zawierała azotu, izoenzymy 1, 3 i 4 wykazywały niewielką aktywność. W korzeniach siewek pobierających jony NH_4^+ aktywność wszystkich izoenzymów była wyższa niż w korzeniach ogórków pobierających jony NO_3^- . Przy braku jonów Ca^{2+} w pożywce azotanowej bądź amonowej stwierdzono podwyższenie aktywności prawie wszystkich izoenzymów, natomiast przy braku K^+ tylko niektórych.