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Polyamine and Ethylene Biosynthesis in Relation to Somatic Embryogenesis in Carrot (*Daucus carota* L.) Cell Cultures

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INTRODUCTION

Carrot cell cultures provide a model experimental system for the analysis of biochemical and molecular events associated with morphogenesis in plants (3, 4, 5, 14). Among the biochemical changes accompanying somatic embryogenesis in this tissue is an increased biosynthesis of polyamines (1, 2, 7, 10, 11, 13). A variety of inhibitors of polyamine biosynthetic enzymes have been used to analyze the role of polyamines in somatic embryogenesis. A summary of our work on the effects of DFMO, DFMA, MGBG and CHAP on somatic embryogenesis, cellular polyamine levels, and activities of ADC, ODC, AdoMet decarboxylase, and AdoMet-synthetase in carrot cell cultures is reported here. Details of materials and methods and results are published elsewhere (6, 8, 9, 12, 13). The results obtained so far support our working hypothesis (7,13) that (a) ethylene is a major suppressor of embryogenesis, and its production is promoted by auxin; and (b) promotion of polyamine biosynthesis through increased utilization of S-adenosylmethionine may adversely affect ACC and ethylene syntheses, which could, in turn, promote somatic embryogenesis.

EFFECTS OF INHIBITORS ON SOMATIC EMBRYOGENESIS

Cells remain undifferentiated in the medium containing 0.1 or 0.5 mg/L 2,4-D. Globular embryos appear within 7 to 10 d after transfer to 2,4-D free medium, and mature embryos are abundant by 14 to 20 d of culture. When 2,4-D is added to cultures after 2, 5, or 10 d of growth in 2,4-D free medium, normal growth of the differentiating embryos ceases within 2 d and callus proliferation begins. If 1 to 10 mM DFMO is added along with 2,4-D, normal development of embryos continues although the growth rate is slower (13). In contrast, 0.1 mM DFMA completely inhibits embryogenesis.

In the presence of MGBG (5 to 500 μ M), development of somatic embryos in the auxin-free medium is effectively blocked at the globular stage (12). In the presence of 0.5 mM CHAP, the development of somatic embryos is only slowed down but not inhibited (6).

EFFECTS OF INHIBITORS ON ENZYME ACTIVITIES

Arginine decarboxylase is the major enzyme for putrescine biosynthesis in this tissue (1, 11, 13). While DFMO promotes ADC activity during the first 10 d, DFMA significantly reduces cellular ADC. ODC activity is very low and remains unchanged during the first 10 to 12 d of culture in all cases. After this time, there is a sharp increase in ODC activity in cultures that produce mature, green somatic embryos (minus 2,4-D and 2,4-D plus DFMO treatments). MGBG significantly inhibits ADC activity both in short-term (2 to 48 h) as well as long-term (1 to 6 d) treatments (12). Although during the first 4 h of treatment with CHAP there is some decrease in ADC activity, beyond 8 h (up to 6 d) the enzyme activity is higher in 0.5 mM CHAP treated cells (6).

The activity of AdoMet synthetase increases 25-fold during the first 5 d of culture both in the presence or the absence of 2,4-D, the activity always being higher in the former (9). DFMO, DFMA, and CHAP have little effect on this enzyme. MGBG strongly inhibits this enzyme. The activity of AdoMet decarboxylase increases during the first 2 d followed by a gradual loss of activity during the next 5 d (9). This enzyme is inhibited by 2,4-D, DFMA, MGBG, and CHAP.

EFFECTS OF INHIBITORS ON CELLULAR POLYAMINES

Cellular putrescine levels are significantly promoted by DFMO. Spermidine, which is the most abundant of the three polyamines, increases up to 6 d both in the presence and the absence of 2,4-D, being significantly higher in the latter. DFMO promotes the cellular concentrations of both spermidine and spermine. Treatment with DFMA results in lower putrescine and spermidine, whereas spermine is slightly promoted (13). Putrescine is higher in the presence of MGBG or CHAP than that in the control cultures. Both spermidine and spermine are inhibited by MGBG (6, 12). While spermidine levels are reduced by CHAP in a concentration-dependent manner, a significant increase in spermine is seen beyond 2 d.

EFFECTS OF INHIBITORS ON ACC AND ETHYLENE

Throughout the 10-d culture period, ACC is higher in the MGBG-treated cells than the controls (12). Cellular ACC is not affected by CHAP. ACC as well as ethylene production are higher in the presence of 2,4-D as compared with minus 2,4-D cultures. DFMO inhibits ethylene production (13).

CONCLUSION

It is apparent that in carrot cell cultures: (a) auxin suppresses somatic embryogenesis, (b) auxin promotes ethylene biosynthesis, (c) ethylene inhibits somatic embryogenesis, (d) development of somatic embryos in the presence of 2,4-D plus DFMO is accompanied by increased polyamine biosynthesis and decreased ethylene production, (e) inhibition of polyamine biosynthesis results in an inhibition of somatic embryogenesis, and (f) the pathway for spermidine and spermine biosynthesis shares (and probably competes for) a common precursor with ethylene biosynthesis. Our results on the effects of inhibitors on cellular polyamine levels are in agreement with the published studies on animal and plant tissues and also support the working hypotheses described earlier (7, 13).

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