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Role of Polyamines in DNA Synthesis of *Catharanthus roseus* Cells Grown in Suspension Culture

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INTRODUCTION

The requirement for polyamines in the proliferation of cells was first demonstrated in bacteria (3). While significant progress has been made in this field using animal cell cultures, only preliminary studies have been reported with plant tissues. Serafini-Fracassini *et al.* (9) showed a marked increase in polyamine synthesis early during the G1 phase, concomitant with the synthesis of RNA, following the break of dormancy in *Helianthus tuberosus* tuber tissue. A second phase of polyamine accumulation began during the progression of S phase. Torrigiani *et al.* (10) and Phillips *et al.* (7) later showed that all major polyamines peaked during the G1 phase. This increase in polyamines was preceded by increased ODC and ADC activities.

An understanding of the mechanism of regulation of cell division requires a biochemical analysis of the progression of the cell cycle. To achieve this it is essential to have a synchronously dividing mass of cells. Although induced synchrony has been reported in suspension cultures of several plant species (4), only a few of them are sufficiently synchronous for biochemical studies. A high degree of synchronization of cell division (up to 80%) in suspension cultures of *Catharanthus roseus* can be induced by phosphate starvation (1). This system has been used extensively to study various aspects of cell cycle such as phase-specific cell wall constituents, polypeptides, and mRNA during cell cycle (2, 5). In this paper we report our preliminary findings on the effects of DFMO, DFMA and MGBG on the (a) DNA synthetic activity; (b) activities of ODC, ADC and AdoMetDC enzymes; and (c) cellular polyamine levels in *C. roseus* cells grown in batch cultures.

MATERIALS AND METHODS

Cell cultures were maintained and synchronized according to the procedure of Kodama *et al.* (5). Enzyme activities and polyamine concentrations were

determined according to the methods of Robie and Minocha (8) and Minocha *et al.* (6). For thymidine incorporation, the cells were incubated in [^{3}H]thymidine (2 μCi/mL) for 30 min, precipitated with 4% (v/v) perchloric acid, washed on GFC filter (Whatman) with perchloric acid, followed by 80% (v/v) ethanol and 100% ethanol, and dried before counting for radioactivity.

RESULTS AND DISCUSSION

Using asynchronous cell suspensions, it was observed that ADC was the predominant enzyme for putrescine biosynthesis. ODC activity was barely detectable and never exceeded 10% of that of ADC. The activity of ADC, cellular putrescine, and DNA synthesis were all inhibited by 0.1 mM DFMA. The inhibition of ADC and DNA synthesis was reversible by the addition of 0.2 mM putrescine to the medium. The activity of ODC, cellular polyamine levels or DNA synthesis were not affected by DFMO. The activities of ADC and AdoMetDC were promoted by 200 to 400% by DFMO. Similar effects of DFMO on ADC have also been observed in suspension cultures of wild carrot (8). Inhibition of AdoMetDC by 0.2 mM MGBG was accentuated by 0.2 mM spermidine within 24 h. MGBG elicited a short-lived inhibition of DNA synthesis at 6 h. By 24 h, cells seemed to recover from the MGBG effect. The extent of inhibition or promotion by these compounds varied significantly with the age of cultures.

Experiments using the synchronous cell cultures to which DFMA was added also showed inhibition of putrescine synthesis and cell division. These results indicate that putrescine plays an important role in cell division in *C. roseus*. Future studies are being directed at elucidating the subcellular localization of the action of putrescine (i.e., cytoplasmic vs. nuclear factors) using synchronized cultures.

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