Translational regulation of ornithine decarboxylase by polyamines

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The activity of ornithine decarboxylase (ODC), the first and rate-limiting enzyme in the polyamine biosynthetic pathway, is dramatically increased in proliferating cells. In addition to transcriptional regulation of ODC, the present study shows that the enzyme is regulated at the translational level by putrescine and spermidine. ODC synthesis is inhibited by an increase and stimulated by a decrease in their cellular content. Spermidine is a more potent negative regulator than is putrescine. The effects of polyamines on ODC synthesis were not attributable to changes in the cellular content of ODC mRNA, thus demonstrating regulation at the translational level.

Ornithine decarboxylase *Translational regulation* *Polyamine* *Putrescine* *Spermidine*

1. INTRODUCTION

The maintenance of adequate intracellular polyamine levels is essential for normal progression through the cell cycle and for a normal course of cell differentiation [1,2]. Accordingly, polyamine biosynthesis is highly regulated. The first reaction in the biosynthetic pathway, the formation of putrescine, is catalyzed by ornithine decarboxylase (ODC). This enzyme appears to be regulated by changes in production and/or turnover of ODC mRNA [3–7], and by changes in turnover of ODC protein [8–11].

In addition to these levels of ODC regulation, we recently obtained indirect evidence for translational control [7]. Thus, Ehrlich ascites tumor cells grown in the presence of ODC inhibitors [α-methylornithine (MO) or α-difluoromethylornithine (DFMO)] were found to exhibit an increased ODC content, despite the loss of ODC activity. The increase was not explained solely by a reduced turnover of the enzyme. Instead our data indicated an increased synthesis of ODC, which was not attributable to a change in the cellular content of ODC mRNA. Taken together our data indicated a regulation at the level of translation. Further support for translational control of ODC was recently obtained in another laboratory using a DFMO-resistant mouse myeloma cell line that overproduces the enzyme due to amplification of an ODC gene [12].

The present study extends our previous findings and provides direct evidence for regulation of ODC at the translational level. We demonstrate that the increase in ODC content (induced by DFMO-mediated putrescine and spermidine depletion) is accompanied by an increased incorporation of [^{35}S]methionine into ODC protein. Furthermore, we show that addition of putrescine or spermidine causes suppression of ODC synthesis, and that spermidine is a more potent negative effector than is putrescine. These changes in ODC synthesis were not due to effects on the cellular content of ODC mRNA.

2. EXPERIMENTAL

2.1. *Materials*

DFMO was a generous gift from the Merrell Dow Research Institute, Strasbourg, France.

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[^{35}S]Methionine and [^{32}P]dCTP were purchased from Amersham. cDNA (pODC 934) encoding mouse kidney ODC was a kind gift from Dr Franklin G. Berger [3].

2.2. *Cells*

Ehrlich ascites tumor cells were grown in suspension culture in a 1:1 mixture of Eagle's minimum essential medium and Ham's F12 medium (lacking putrescine) supplemented with 0.2% bovine serum albumin and antibiotics [7]. The cells were seeded at a density of 1.0×10^5 cells/ml in the absence or presence of polyamines (1 μM–10 mM putrescine or spermidine) or DFMO (5 mM). After 24 h the cells were harvested and analyzed for ODC activity, ODC synthesis or ODC mRNA content.

2.3. *ODC activity*

Extracts for measurement of ODC activity were prepared by sonicating the cells in 0.1 M Tris-HCl (pH 7.5) containing 0.1 mM EDTA and 2.5 mM dithiothreitol, followed by centrifugation at $20000 \times g$ for 20 min. ODC activity was assayed as described [7].

2.4. *ODC synthesis*

The rate of ODC synthesis was determined by measuring the incorporation of [^{35}S]methionine into the enzyme. The cells were reseeded at a density of 1.0×10^6 cells/ml in methionine-free Eagle's minimum essential medium supplemented with 10 μCi/ml of [^{35}S]methionine. Incubation was carried out at 37°C for 25 min, whereupon the cells were harvested and sonicated as described above. After centrifugation at $30000 \times g$ for 30 min, aliquots of the supernatants (containing equal amounts of acid-insoluble radioactivity) were incubated with a monospecific antibody against mouse kidney ODC [13] in the presence of 0.1% bovine serum albumin, 0.1% Triton X-100 and 0.1% SDS for 30 min at room temperature. To ascertain precipitation of all ODC protein, a 100-fold excess of ODC antibody was used [13]. Precipitation of the antibody-ODC complex was achieved using protein A adsorbent (*Staphylococcus aureus*). After thorough washing in 10 mM Tris-HCl (pH 7.5) containing 0.1 mM EDTA, 0.5 mM dithiothreitol, 0.1% bovine serum albumin, 0.1% Triton X-100 and 0.1% SDS, the immunoprecipitates were processed for SDS-polyacrylamide slab gel electrophoresis essentially as in [14]. Radioactivity in the gels was visualized by autoradiography after treatment with Amplify (Amersham).

2.5. *ODC mRNA content*

For analysis of ODC mRNA, total RNA was isolated by the guanidinium/cesium chloride method [15], fractionated in a formaldehyde-containing 1% agarose gel [16], transferred onto Gene-Screen (New England Nuclear) and hybridized to pODC 934 labeled with [^{32}P]dCTP [17]. The relative amounts of ODC mRNA were measured by cutting out the bands and counting their radioactivities in a liquid scintillation spectrometer.

3. RESULTS AND DISCUSSION

When Ehrlich ascites tumor cells are stimulated to grow and proliferate by dilution in fresh medium, there is a dramatic increase in the activity of ODC. This enzyme is regulated by many different mechanisms, in which putrescine (the product) and spermidine (the subsequent polyamine in the biosynthetic pathway) seem to have important roles. Accordingly, addition of putrescine or spermidine to the growth medium at the time of dilution was found to abolish the induction of ODC activity (assayed after 24 h) in Ehrlich ascites tumor cells (fig.1). Spermidine was consistently more effective than putrescine, causing greater than 50% inhibition of the ODC activity at a concentration as low as 1 μM (fig.1).

To determine whether the polyamines act directly on ODC protein synthesis, the Ehrlich ascites tumor cells were pulse-labeled with [^{35}S]methionine after 24 h in the presence of various concentrations of putrescine or spermidine. ODC protein was then immunoprecipitated and analyzed by gel electrophoresis and autoradiography. Fig.2 shows that micromolar concentrations of the polyamines effectively suppressed the incorporation of [^{35}S]-methionine into the enzyme. Again, spermidine was found to be more effective than putrescine.

The suppression of ODC synthesis caused by the polyamines was not a result of a decrease in ODC mRNA content, as determined by hybridization of total cellular RNA to an ODC cDNA clone (fig.3). Therefore, it may be concluded that polyamines

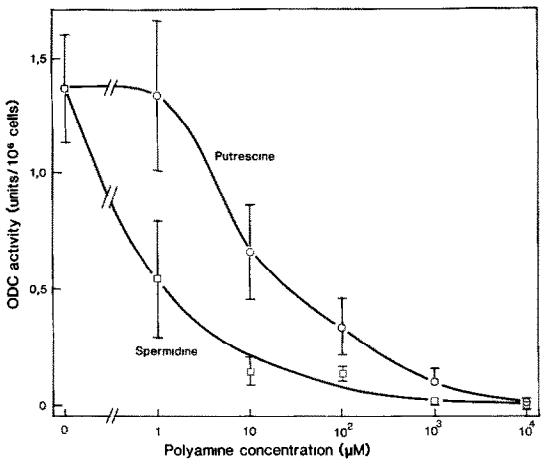

Fig.1. Effects of putrescine and spermidine on the induction of ODC activity in Ehrlich ascites tumor cells. The polyamines were added at the time of growth stimulation, and the ODC activity was determined 24 h later.

exert a negative feedback control of ODC expression at the level of translation. It should be pointed out, however, that although the ODC activity was eradicated at high polyamine concentrations (1–10 mM), ODC synthesis was not completely blocked (figs 1,2). Hence, it appears that, in addition to their translational control of ODC expression, the polyamines may affect the turnover of ODC protein. This contention is supported by other studies, in which 10 mM putrescine was found to produce a significant acceleration of ODC degradation [10,11].

That polyamines act as negative regulators of ODC translation is consistent with our previous finding that depletion of the cellular polyamine content, by addition of an ODC inhibitor to the growth medium, resulted in a compensatory increase in cellular content of ODC, which was not attributable to changes in ODC mRNA content or ODC turnover [7]. That the synthesis of ODC protein is indeed stimulated by DFMO-mediated putrescine and spermidine depletion is demonstrated by an elevated incorporation of [^{35}S]methionine into the enzyme (fig.2B, lane 2).

Translational control of ODC is suggested also by short-term experiments using a DFMO-resistant mouse myeloma cell line that overproduces the enzyme [12]. Addition of a very high (30 mM) concentration of putrescine was required to obtain maximal inhibition of ODC synthesis in these cells.

The translational control of ODC expression is particularly interesting in view of the fact that

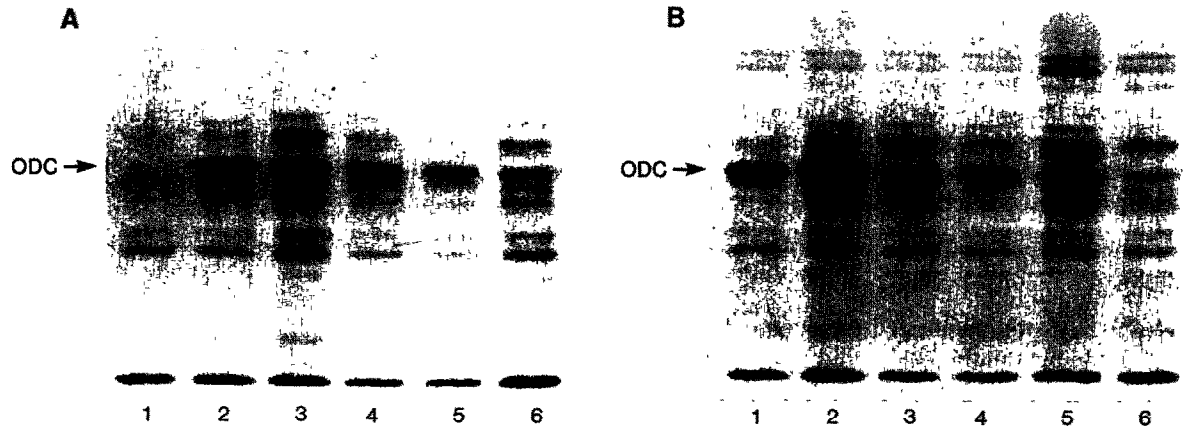

Fig.2. Effects of putrescine (A) and spermidine (B) on ODC synthesis in Ehrlich ascites tumor cells. The polyamines or DFMO were added at the time of growth stimulation, and the incorporation of [^{35}S]methionine into ODC protein (precipitated with a 100-fold excess of monospecific antibody [13]) was determined during a 25 min pulse 24 h later. (A) Lane 1, untreated control (24 h), non-immune serum. Lane 2, untreated control (24 h), ODC antiserum. Lanes 3–6, treatment with putrescine (10^1, 10^2, 10^3 and 10^4 μM, respectively). (B) Lane 1, untreated control (24 h), ODC antiserum; lane 2, treatment with DFMO (5 mM). A band with slightly lower M_r than the major form of ODC is also seen, probably representing a degradation product [14]. Lanes 3–6, treatment with spermidine (10^1, 10^2, 10^3 and 10^4 μM, respectively). Arrow, migration of [^{3}H]DFMO-labeled purified mouse kidney ODC (~53 kDa).

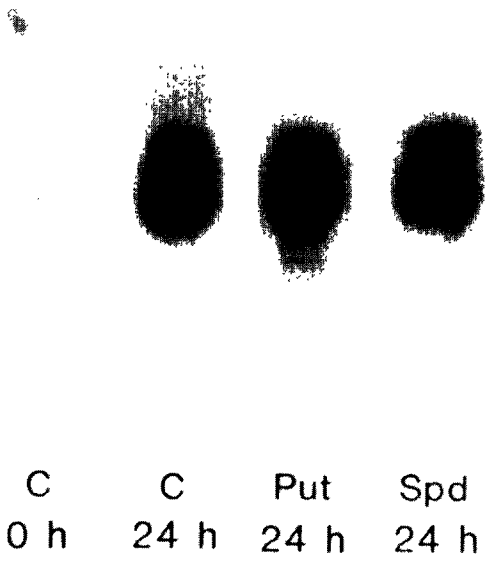

Fig.3. Effects of polyamines on the ODC mRNA level in Ehrlich ascites tumor cells. Putrescine (Put, 10 mM) or spermidine (Spd, 1 mM) was added at the time of growth stimulation. The ODC mRNA content was determined at 0 h and after 24 h of growth in the absence or presence of the polyamines. C, control. The relative amounts of ODC mRNA were 1:10.2:11.8:11.8.

ODC mRNA has a long 5′-non-coding leader containing four initiator codons [18]. It is conceivable that high polyamine concentrations unmask the initiator codons, thus causing inefficient translation of the proper sequence [12,19].

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