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Feedback regulation of ornithine decarboxylase expression

Studies using a polysomal run-off system

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The rate-controlling enzyme in polyamine synthesis, ornithine decarboxylase (ODC), is subject to feedback regulation by the polyamines at the level of translation. In the present study we used a cell-free translation system to further investigate the mechanism by which this regulation occurs. Lysates of ODC-overproducing cells were capable of synthesizing large amounts of ODC. The degree of initiation was poor in the lysates and the synthesis of ODC was mainly a result of continued elongation of peptide chains on pre-initiated ribosomes. By determining the amount of ODC produced in the lysate, we obtained an estimate of the number of ribosomes that were actively translating ODC mRNA at the moment of lysis. Using this polysomal run-off assay we demonstrated that the polyamine-mediated regulation of ODC synthesis occurs without any change in the number of ribosomes associated with the message. This finding indicates that the polyamines exert a coordinate effect on initiation and elongation.

Ornithine decarboxylase; Polyamine; Translational regulation

1. INTRODUCTION

Cell growth and differentiation are dependent on an adequate supply of the polyamines putrescine, spermidine and spermine [1,2]. The first and often rate-limiting step in polyamine synthesis is catalyzed by ornithine decarboxylase (ODC), which has the fastest turnover among mammalian enzymes [3]. The importance of the polyamines is reflected in the extensive control of their synthesis. ODC is subject to a strong feedback regulation by the polyamines [4–9]. Thus, a cellular excess of polyamines results in a rapid decrease in ODC activity, whereas a reduction of the polyamine content induces a rise in ODC activity. Part of these changes may be due to changes in the turnover of the ODC protein [1,2]. However, the major part is due to changes in the synthesis rate of the enzyme [4–9].

The polyamine-mediated control of ODC synthesis is not carried out at the level of transcription, because no changes are seen in the steady-state level of ODC mRNA [4–9]. Instead, it appears that the regulation is exerted at the translational level. The mechanism(s) behind this regulation is not yet known. ODC mRNA has a very long 5′ untranslated region, which has been suggested to be involved in the translational control of the enzyme [10,11]. This would imply that the control of ODC synthesis is mainly exerted at the initiation stage. In the present study we have used an in vitro polysomal run-off system to investigate this possibility.

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2. EXPERIMENTAL

2.1. *Materials*

The ODC inhibitor 2-difluoromethylornithine (DFMO) was a generous gift from the Merrell Dow Research Institute, Cincinnati, OH. [^{35}S]Methionine was obtained from Amersham. Aurintricarboxylic acid and 7-methylguanosine 5′-triphosphate were purchased from Sigma and Pharmacia, respectively.

2.2. *Lysates*

An ODC-overproducing mouse L1210 leukemia cell line (L1210-DFMO^r) was used for preparation of lysates. The cells were routinely grown in the presence of 20 mM DFMO in RPMI 1640 medium containing 10% fetal calf serum and antibiotics [9]. In some experiments the cells were grown in the absence of DFMO for 4–6 passages before being used for preparation of lysates. Cell lysates were prepared one day after seeding as described by Clemens [12] with the modification that spermidine was omitted from the lysis buffer.

2.3. *ODC synthesis*

Synthesis of ODC was determined by measuring the incorporation of [^{35}S]methionine into the enzyme. Translation of the endogenous mRNAs in the lysates was carried out in the presence of 0.1 M KCl, 0.8 mM $MgCl_2$, 1.0 mM ATP, 0.2 mM GTP, 10 mM creatine phosphate, 0.25 mg/ml creatine phosphokinase, 1.0 mM glucose, 0.2 mM dithiothreitol, 50 μM of a methionine-free amino acid mixture, 20 μCi [^{35}S]methionine and 0–2.4 mM spermidine for 10 min (except for the time course study) at 30°C. ODC was immunoprecipitated

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and fractionated by SDS-PAGE [7,13]. The migration of ODC was determined using pure mouse ODC $M_r \approx 53\,000$) labelled with [^{3}H]DFMO.

2.4. *Polyamine content*

The content of polyamines in the lysates was measured as previously described [14] using an amino acid analyzer (Biotronik LC 5001).

3. RESULTS AND DISCUSSION

Studies of ODC synthesis are hampered by the fact that the cellular content of ODC is usually extremely low [2]. To overcome this obstacle, we have used an ODC-overproducing cell line (L1210-DFMO^r) in which ODC represents 4–5% of the soluble protein synthesized [9]. This cell line was isolated by selection for resistance to the antiproliferative effect of the ODC inhibitor DFMO. The high expression of ODC in these cells is caused by amplification of the ODC gene, with a concomitant increase in the steady-state level of the mRNA [9]. In spite of the 100-fold increase in expression, ODC is still subject to feedback control by the polyamines [9]. When the L1210-DFMO^r cells, which are routinely maintained in a medium containing 20 mM DFMO, are seeded in a medium lacking DFMO their synthesis of ODC is reduced 10-fold [9]. However, this is not related to a loss of the amplification because the high expression rate is rapidly restored by addition of DFMO. Instead the decrease in ODC synthesis appears to be related to an increase in cellular polyamine synthesis and content caused by the withdrawal of DFMO [9]. The polyamine-mediated change in ODC synthesis is not associated with any change in the steady-state level of ODC mRNA and thus resembles that seen in other cells [9]. Hence, the L1210-DFMO^r cells are extremely useful for studies of the feedback control of ODC. The synthesis of ODC as well as the content of ODC mRNA are high enough to be easily determined. Moreover, by growing the cells in the presence or absence of DFMO it is possible to change the rate of ODC synthesis by more than 10-fold (without changing the ODC mRNA level or the synthesis of other proteins).

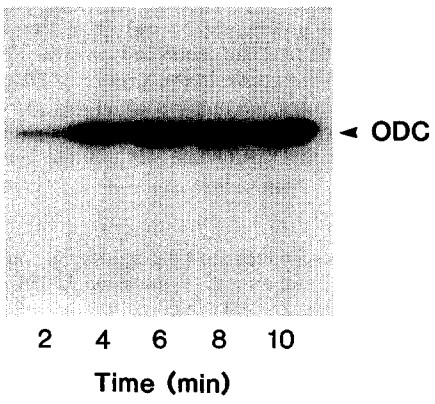

Fig.1. Time course of ODC synthesis in a lysate prepared from L1210-DFMO^r cells grown in the absence of DFMO.

ODC mRNA contains a very long 5′ untranslated region which has been suggested to be involved in the translational regulation of ODC [10,11]. This sequence is highly GC-rich and may easily form strong secondary structures. It is conceivable that formation of secondary structures in this region interferes with the rate of initiation and that polyamines directly or indirectly affect this formation.

A change in the rate of initiation would give rise to a change in the number of ribosomes actively translating the ODC mRNA at any given moment. To determine whether the polyamine-mediated reduction in ODC synthesis was due to a decrease in the number of ribosomes translating the message we used an in vitro polysomal run-off assay. In this assay we took advantage of the fact that lysates made from mammalian cells, other than reticulocytes, do not initiate protein synthesis very well [12]. Most lysates, however, continue elongation of initiated nascent peptide chains to full-size products. By determining the amount of the specific product (ODC) synthesized in the lysate, we obtained a measurement of the number of ribosomes that were actively translating the specific mRNA at the moment of lysis.

Lysates were prepared from L1210-DFMO^r cells and assayed for ODC synthesizing capacity using [^{35}S]methionine labelling and immunoprecipitation. As shown in fig.1 the lysates were capable of synthesizing fairly large amounts of full-length ODC. No significant amounts of smaller labelled polypeptides were precipitated with the antibody. This indicates that no premature termination was taking place in the lysate. The synthesis of ODC (as well as that of total protein) exhibited a maximal rate for less than 4 min, suggesting a poor degree of initiation. That the protein synthesis in the lysates was mainly due to elongation by preinitiated polysomes was confirmed using two different inhibitors of initiation: 7-methylguanosine 5′-triphosphate and aurintricarboxylic acid. No effect on protein synthesis was seen when these inhibitors were added to the lysate in concentrations well above those needed for blocking initiation (results not shown).

When assayed for protein synthetic capacity the lysate gave much better results when prepared from cells grown in the absence of DFMO than when prepared from cells grown in the presence of DFMO. As shown in table 1, the lysates made from cells grown

Table 1

Polyamine concentrations in lysates prepared from L1210-DFMO^r cells grown in the absence or presence of DFMO (20 mM)

Lysate	Putrescine[a]	Spermidine[a]	Spermine[a]
+ DFMO	5 ± 3	106 ± 15	187 ± 39
− DFMO	859 ± 96	438 ± 98	140 ± 50

[a]μM; mean ± S.E.M., n = 4

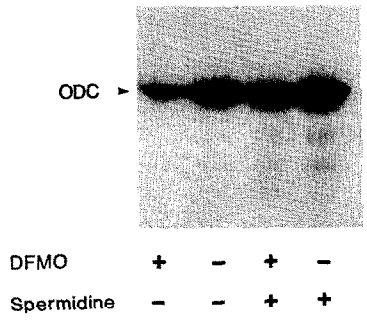

Fig.2. Effect of spermidine on ODC synthesis in a lysate prepared from L1210-DFMO^r cells grown in the presence of DFMO (20 mM).

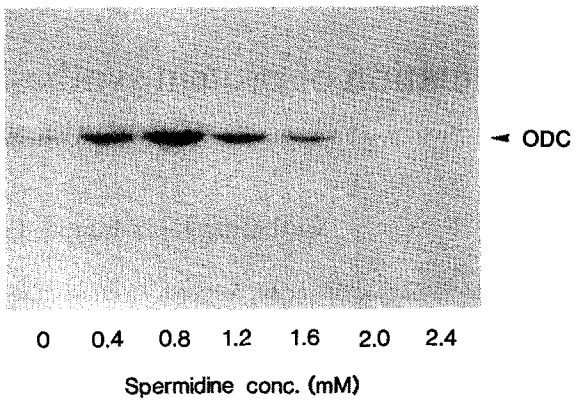

Fig.3. Effects of spermidine (0.8 mM) on ODC synthesis in lysates prepared from L1210-DFMO^r cells grown in the absence or presence of DFMO (20 mM).

in the absence of DFMO contained much more putrescine and spermidine than those made from cells grown in the presence of the inhibitor. This probably only reflects the presence of a larger amount of enzymatically active ODC in the untreated cells [9]. The capacity of the lysate to synthesize proteins, including ODC, was strongly dependent on the concentration of spermidine in the lysate. Optimal effects were obtained with a spermidine concentration between 0.8 and 1.0 mM (fig.2). When the spermidine concentrations of the lysates were made equal, and optimal, about the same protein synthetic capacity was seen whether the lysates were prepared from cells grown in the absence or presence of DFMO. Similarly, there was no major difference in the amount of ODC synthesized by the two different lysates when the spermidine concentrations were equalized (fig.3). This finding indicates that the decrease in ODC synthesis, seen in the L1210-DFMO^r cells after withdrawal of DFMO from the medium [8], is not caused by a reduction in the number of ribosomes that are associated with the mRNA. A similar conclusion was drawn from studies of ODC mRNA distribution in polysome profiles ([15] and Stjernborg et al., unpublished observation). In these studies, it was demonstrated that polyamine-mediated changes in ODC synthesis occur without a significant change in the distribution of ODC mRNA in polysome profiles (which reflects the number of ribosomes associated with each ODC message). However, since the major part of the ODC mRNA was found in a region containing ribosomal subunits and monosomes [15] even a minor change in the distribution of ODC mRNA in the polysome profile may reflect a large change in the total number of ribosomes associated with the ODC mRNA. The technique used in the present study, on the other hand, gives a more direct estimate of the amount of ribosomes that are translating the message.

Taken together, our data indicate that the polyamines regulate ODC synthesis not merely by affecting the rate of initiation. Apparently, there is a corresponding change in the elongation rate, because the effects of the polyamines on ODC synthesis are not correlated with a change in the number of ribosomes associated with each ODC mRNA. The exact mechanism by which this regulation occurs remains elusive and more work is obviously needed for its full clarification.

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