Regulation of *S*-adenosylmethionine decarboxylase by polyamines in Ehrlich ascites-carcinoma cells grown in culture

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1. The mechanism of stimulation of *S*-adenosylmethionine decarboxylase (EC 4.1.1.50) activity by inhibitors of ornithine decarboxylase (EC 4.1.1.17), namely DL- α -difluoromethylornithine, 1,3-diaminopropane and 1,3-diaminopropan-2-ol, was studied in Ehrlich ascites-tumour cells grown in suspension cultures. 2. Difluoromethylornithine and diaminopropane, although decreasing the content of putrescine and spermidine, markedly stimulated adenosylmethionine decarboxylase activity after exposure of the cells to the drugs for 8 h, whereas the effect of diaminopropanol only became apparent many hours later. In tumour cells exposed to any of the inhibitors, a close negative correlation existed between the activity of adenosylmethionine decarboxylase and the intracellular concentration of spermidine and/or spermidine plus spermine, suggesting that a depletion of higher polyamines triggered enhancement of adenosylmethionine decarboxylase activity. 3. The mechanism of difluoromethylornithine- and diaminopropane-induced stimulation of adenosylmethionine decarboxylase involved (a) a marked increase in the apparent half-life of the enzyme and (b) an induction of enhanced enzyme synthesis. Diaminopropanol seemed to act solely via an induction mechanism. 4. The increased adenosylmethionine decarboxylase activity elicited by difluoromethylornithine could be restored to control values by micromolar concentrations of exogenous spermidine and spermine in 4 h and by putrescine in 22 h. In addition to the natural polyamines, elevated adenosylmethionine decarboxylase activity could be repressed by 3,3'-iminodipropylamine, a close analogue of spermidine, but not by non-physiological diamines. 5. Addition of spermidine and actinomycin D to cultures treated with difluoromethylornithine produced a comparable decay of enhanced adenosylmethionine decarboxylase activity (with an apparent half-life of about 2.5 h), whereas the effect of cycloheximide was much more rapid. The present results suggest that polyamines may regulate adenosylmethionine decarboxylase at the transcriptional level of gene expression.

Increasing experimental evidence seems to indicate that undisturbed polyamine synthesis is essential for normal cell proliferation (for references see Jänne *et al.*, 1978). In order to elucidate the physiological function of the naturally occurring polyamines putrescine, spermidine and spermine, numerous efforts have been made to produce cellular depletion of these polyamines. However, most inhibitors of polyamine biosynthesis induce compensatory changes which make the achievement of total polyamine deficiency difficult. Substrate analogues, most of which are reversible competitive inhibitors of the key enzymes ornithine decarboxylase and adenosylmethionine decarboxylase, stabilize the enzymes against intracellular proteolysis (Pegg *et al.*, 1973; Harik *et al.*, 1974; McCann *et*

al., 1977). On the other hand, diamine repressors, which are indirect inhibitors of ornithine decarboxylase, may take over the function of natural polyamines, either as such or by serving as substrates in the propylamine transferase reaction to yield analogues of higher polyamines (Hibasami & Pegg, 1978; Mamont *et al.*, 1978b; Hölttä *et al.*, 1979b). Moreover, diamines may activate pre-existing adenosylmethionine decarboxylase (Alhonen-Hongisto *et al.*, 1979a). Despite its polyamine-depleting properties, methylglyoxal bis(guanyldihydrazone), a potent inhibitor of eukaryotic adenosylmethionine decarboxylase (Williams-Ashman & Schenone, 1972), exerts a number of effects apparently unrelated to polyamine metabolism (Hölttä *et al.*, 1973; Newton & Abdel-Monem, 1977; Dave *et*

al., 1978; Hölttä *et al.*, 1979a) and seems to compete with polyamines for a common transport system (Mihich *et al.*, 1974; Seppänen *et al.*, 1980).

Only after the discovery of irreversible inhibitors of ornithine decarboxylase could a more specific inhibition of polyamine synthesis be achieved. DL- α -Difluoromethylornithine, an enzyme-activated irreversible inhibitor of ornithine decarboxylase (Metcalf *et al.*, 1978), effectively depleted cells or tissues of putrescine and spermidine (Mamont *et al.*, 1978a; Danzin *et al.*, 1979). A striking depression of DNA synthesis, as a consequence of polyamine deprivation, was seen in Ehrlich ascites cells *in vivo* as well as in culture (Alhonen-Hongisto *et al.*, 1979a,b), in accordance with the results obtained by Mamont *et al.* (1978a). In most cases spermine concentrations remained unchanged, which suggested an incomplete inhibition of ornithine decarboxylase.

The finding that difluoromethylornithine stimulated adenosylmethionine decarboxylase in rat hepatoma cells (Mamont *et al.*, 1978b) and in spleens of mice inoculated with L1210 leukaemia cells (Prakash *et al.*, 1978) revealed a further intracellular compensatory mechanism by which cells exposed to the drug could resist polyamine deprivation. In fact, the undisturbed spermine accumulation could also be explained in terms of a marked stimulation of adenosylmethionine decarboxylase, which could supply decarboxylated adenosylmethionine for the synthesis of spermine.

The effect of difluoromethylornithine, diaminopropane and diaminopropanol, all inhibitors of mammalian ornithine decarboxylase, on adenosylmethionine decarboxylase has now been studied by using cultured Ehrlich ascites cells as the test model. While profoundly inhibiting the accumulation of putrescine and spermidine, these inhibitors produced a time-dependent stimulation of adenosylmethionine decarboxylase activity, the extent of which inversely correlated with the intracellular concentrations of polyamines. As also noticed by Mamont *et al.* (1978b), the stimulated enzyme activity could be repressed by spermidine and spermine and less effectively by putrescine. The mechanism of the stimulation appeared to involve a moderate stabilization and an enhanced synthesis of the enzyme. The regulation exerted by polyamines on adenosylmethionine decarboxylase appeared to take place at the transcriptional level of gene expression, as judged by the similar response of difluoromethylornithine-stimulated enzyme activity to spermidine and actinomycin D.

Experimental

Cell cultures

Ehrlich ascites-carcinoma cells were maintained in

the peritoneal cavity of female albino mice (weighing about 30g) by weekly inoculations. The cells were adapted to growth in suspension cultures during 6 weeks with frequent changes of medium (RPMI 1640; Orion Diagnostica, Helsinki, Finland) supplemented with 10% (v/v) pooled human serum (Finnish Red Cross Transfusion Service, Helsinki, Finland), 2 mM-glutamine and both streptomycin and penicillin (50 μ g/ml). After the period of adaptation, the doubling time of the cells was about 14 h. Stock cultures were maintained in 75 cm² Falcon flasks and experimental cultures were grown in 30 ml sterile containers incubated horizontally at 37°C.

Preparation of cell extracts

The ascites cells were harvested by low-speed centrifugation (2000g, 3 min) and suspended in 25 mM-Tris/HCl buffer (pH 7.4) containing 0.1 mM-EDTA and 1 mM-dithiothreitol. The cells were disintegrated ultrasonically with a Branson sonifier (3 $\times$ 5 s). The homogenates were centrifuged for 25 min at 100 000g_{max}, and the resulting cytosol fractions were used for the assay of adenosylmethionine decarboxylase activity.

Chemicals

S-Adenosyl-L-[1-¹⁴C]methionine was prepared as described by Pegg & Williams-Ashman (1969). Putrescine, spermidine, spermine and cadaverine were purchased from Calbiochem (San Diego, CA, U.S.A.). 1,6-Diaminohexane was a product of BDH Chemicals (Poole, Dorset, U.K.). 1,3-Diaminopropane, 1,3-diaminopropan-2-ol and 1,7-diaminohexane were obtained from Fluka A.G. (Buchs SG, Switzerland) and 1,4-diaminobut-2-ene was from K & K Laboratories (Plainview, NY, U.S.A.). 3,3'-Iminodipropylamine (3,3'-diaminodipropylamine) and actinomycin D were purchased from Aldrich-Europe (Beerse, Belgium). Cycloheximide was a product of Nutritional Biochemical Corp. (Cleveland, OH, U.S.A.). DL- α -Difluoromethylornithine was a generous gift from the Centre de Recherche Merrell International (Strasbourg, France).

All compounds were neutralized and sterilized before use.

Analytical methods

The activity of adenosylmethionine decarboxylase was assayed in the presence of 2.5 mM-putrescine by the published method (Jänne & Williams-Ashman, 1971). Polyamines were determined after dansylation (5-dimethylaminonaphthalene-1-sulphonylation) by the method of Seiler (1970) with the modifications introduced by Dreyfuss *et al.* (1973). Cellular content of DNA was measured by the method of Giles & Myers (1965) and protein by the method of Lowry *et al.* (1951), with bovine serum albumin as the standard. Cell densities were

measured in an electronic particle counter (Coulter Electronics, Harpenden, Herts., U.K.) and the viability of the cells was tested by the dye-exclusion test (Black & Berenbaum, 1964).

The regression lines of enzyme activities in Figs. 2, 3 and 5 were computed by the least-squares method.

The results presented were obtained in single experiments. The reproducibility of the results is highly satisfactory under standard culture conditions.

Results

Stimulation of adenosylmethionine decarboxylase by difluoromethylornithine, diaminopropane and diaminopropanol

When Ehrlich ascites cells were grown in the presence of inhibitors of ornithine decarboxylase, a striking time-dependent stimulation of adenosylmethionine decarboxylase activity was evident, as illustrated in Fig. 1. Difluoromethylornithine and diaminopropane exerted similar effects on enzyme activity up to 20h, after which difluoromethylornithine was more powerful in stimulating the enzyme activity. The action of diaminopropanol on the enzyme activity did not become clearly apparent until 48h (Fig. 1).

Diaminopropanol has no influence on adenosylmethionine decarboxylase *in vitro*, whereas diaminopropane is known to activate the enzyme from Ehrlich ascites cells, although to a much lesser extent than does putrescine (Alhonen-Hongisto *et al.*, 1979a). However, the enzyme activity was always measured in the presence of saturating (2.5 mM) concentrations of putrescine, thus excluding any activation *in vitro* by the diamines.

In comparison with difluoromethylornithine, diaminopropane and diaminopropanol were equally potent in inhibiting total polyamine accumulation

during the first 24h of culture, which likewise was reflected in a similar decrease in cell proliferation, as judged by cell number and accumulation of protein and DNA (results not shown). Of the three inhibitors, difluoromethylornithine produced most complete inhibition of putrescine and spermidine accumulation (Table 1). No marked changes in cellular spermine concentrations were seen until 48h (results not shown).

Fig. 1. Effect of difluoromethylornithine, diaminopropane and diaminopropanol on adenosylmethionine decarboxylase in cultured Ehrlich ascites cells
The cells (initial density $0.15 \times 10^6/\text{ml}$) were grown in the absence (●) or presence of 6 mM-difluoromethylornithine (○), 3 mM-diaminopropane (△) or 3 mM-diaminopropanol (□). The enzyme activities were measured at the time points indicated and expressed as nmol of CO_2 released/mg of protein.

Table 1. Effect of difluoromethylornithine, diaminopropane and diaminopropanol on polyamine accumulation in Ehrlich ascites cells

Experimental details were as in Fig. 1. Polyamine concentrations are expressed as nmol/mg of DNA.

Time (h)	Putrescine			Spermidine			
	Control	Difluoromethylornithine	Diamino-propanol	Control	Difluoromethylornithine	Diamino-propane	Diamino-propanol
8	112	10.4	6.5	505	217	239	282
12	306	6.6	5.2	587	158	123	254
16	670	2.8	2.5	576	63	168	157
20	929	3.1	8.3	586	39	160	173
24	723	1.3	4.4	481	27	141	127
28	1023	3.2	13.8	604	27	87	128
48	620	2.3	6.3	500	32	157	111

Relation of adenosylmethionine decarboxylase activity to intracellular polyamine concentrations

As it seemed evident that increased adenosylmethionine decarboxylase activity in cells treated with difluoromethylornithine, diaminopropane and diaminopropanol was related to polyamine deprivation, the correlation between the enzyme activity and polyamine concentration was studied. The most striking correlation was found between the activity of adenosylmethionine decarboxylase and total concentration of higher polyamines (spermidine plus spermine), as also the concentration of spermine was decreased during 48 h exposure of the cells to the drugs, with a correlation coefficient of -0.89 (Fig. 2). A slightly less pronounced correlation existed between the enzyme activity and spermidine concentration (correlation coefficient -0.85). It thus appears as if the stimulation of adenosylmethionine decarboxylase by various inhibitors of ornithine decarboxylase was a general response of the cells provoked by falling concentrations of higher polyamines, most notably spermidine. This indicates that polyamines obviously have an important regulatory role in expression of adenosylmethionine decarboxylase.

Mechanism of action of difluoromethylornithine, diaminopropane and diaminopropanol on adenosylmethionine decarboxylase

Inhibition of protein synthesis by cycloheximide caused a rapid decay of adenosylmethionine decarboxylase activity in control cells, with an apparent half-life of 50 min, as well as in cultures exposed

Fig. 2. Correlation between adenosylmethionine decarboxylase activity and polyamine concentration (spermidine + spermine) in Ehrlich ascites cells treated with various inhibitors of ornithine decarboxylase

Experimental details and symbols are as in Fig. 1. Polyamine concentrations are expressed as nmol/mg of DNA.

to diaminopropanol (half-life 49 min) (Fig. 3), whereas diaminopropane (half-life 67 min) and especially difluoromethylornithine (half-life 102 min) appeared to protect the enzyme from normal intracellular degradation (Fig. 3). Thus introduction of diaminopropane and difluoromethylornithine to cell cultures resulted in a distinct stabilization of adenosylmethionine decarboxylase. Again, the activation of the enzyme by diamines *in vitro* was excluded by using 2.5 mM-putrescine in the assay system.

As could be expected, the extent of stabilization of adenosylmethionine decarboxylase appeared to be related to the increased enzyme activity (control, 0.43, diaminopropanol, 0.83, diaminopropane, 2.25, and difluoromethylornithine, 4.75 nmol/mg of protein).

Whether the stabilization of adenosylmethionine decarboxylase was the sole mechanism resulting in increased enzyme activity, or whether enhanced enzyme synthesis was also involved, was determined from enzyme-turnover kinetics. According to the equation presented by Schimke & Doyle (1970), the rate constant of enzyme synthesis is directly

Fig. 3. Effect of difluoromethylornithine, diaminopropane and diaminopropanol on the half-life of adenosylmethionine decarboxylase in Ehrlich ascites cells. The cells (initial density 0.15×10^6 /ml) were grown in the absence (●, $t_{1/2} = 50$ min) or presence of 6 mM-difluoromethylornithine (○, $t_{1/2} = 102$ min), 3 mM-diaminopropane (△, $t_{1/2} = 67$ min) or 3 mM-diaminopropanol (□, $t_{1/2} = 49$ min) for 24 h. Adenosylmethionine decarboxylase activity was assayed after an exposure of the cultures to cycloheximide ($10 \mu\text{g}/\text{ml}$) for 0–240 min. At each time point enzyme activities are given relative to the respective activity at the moment of addition of cycloheximide. The fact that the lines do not intersect at the same experimental point at zero time is due to the fit of the lines by the least-squares method.

proportional to the amount of the enzyme and inversely proportional to the half-life of the enzyme when changes of activity with regard to time are relatively slow or time periods employed are much longer than the half-life. By using the values for half-lives given above, rate constants of enzyme synthesis were calculated. Exposure of the cells to difluoromethylornithine, diaminopropane and diaminopropanol for 24 h resulted respectively in 5.2-, 3.9- and 2.0-fold increases in adenosylmethionine decarboxylase synthesis compared with that in control conditions. Thus induction of enhanced enzyme production was undoubtedly involved in all cases. Moreover, with diaminopropanol, as no stabilization was found, induction was the only mechanism responsible for the stimulation of adenosylmethionine decarboxylase.

When a single drug was considered, the relative contribution of the two processes to the enhancement of the enzyme activity seemed to be fairly constant, as judged by the results obtained from difluoromethylornithine-treated cultures with different half-lives of adenosylmethionine decarboxylase (results not shown). This supports the view that both processes are co-ordinately controlled by polyamines.

Repression of elevated adenosylmethionine decarboxylase activity by polyamines

Further support to the idea that adenosylmethionine decarboxylase in difluoromethylornithine-treated cells was regulated by polyamines was obtained from the experiment presented in Fig. 4. When added to cultures exposed to difluoromethylornithine, 10 μ M-spermidine and spermine decreased the elevated enzyme activity to the control value in 4 h (Fig. 4a). A similar decrease was achieved by putrescine only after 22 h, although there was a considerable decrease in the enzyme activity already at 4 h (Fig. 4a). In contrast with the former results, polyamines could not fully normalize increased adenosylmethionine decarboxylase activity in cells treated with diaminopropane (Fig. 4b). The best effect was obtained with spermine, which decreased the enzyme activity 53% in 22 h (Fig. 4b). The incomplete effect of polyamines in restoring the elevated adenosylmethionine decarboxylase in cells exposed to diaminopropane is attributable to the fact that diaminopropane interfered with cellular uptake of natural polyamines. In fact, in the presence of diaminopropane extracellular polyamines only marginally increased the intracellular polyamine concentrations (results not shown).

Fig. 4. Effect of polyamines on adenosylmethionine decarboxylase stimulated by difluoromethylornithine (a) and diaminopropane (b)

Ehrlich ascites cells were diluted to an initial density of 0.16×10^6 /ml and grown in the presence of 6 mM-difluoromethylornithine (a) or 3 mM-diaminopropane (b) for 24 h. The cultures were then supplemented with 10 μ M-putrescine (O), -spermidine (Δ) or -spermine ($\square$) and the enzyme activities measured at indicated time points; ●, no amines added; ▲, control.

Table 2. *Effect of various amines on adenosylmethionine decarboxylase activity stimulated by difluoromethylornithine* Ehrlich ascites cells (initial density $0.11 \times 10^6/\text{ml}$) were grown in the absence or presence of 6 mM-difluoromethylornithine (DFMO) for 40h. At this time difluoromethylornithine-treated cultures were supplemented with $10 \mu\text{M}$ amines and the enzymes activities were measured 4 h later.

Treatment	Adenosylmethionine decarboxylase (nmol/mg of protein)
None	0.66 (18%)
DFMO	3.54 (100%)
DFMO + putrescine	2.76 (80%)
DFMO + 1,4-diaminobutene	2.95 (83%)
DFMO + 1,5-diaminopentane	3.56 (101%)
DFMO + 1,6-diaminohexane	3.70 (105%)
DFMO + 1,7-diaminoheptane	4.11 (116%)
DFMO + 3,3'-iminodipropylamine	1.15 (32%)
DFMO + spermidine	1.55 (44%)
DFMO + spermine	1.14 (32%)

Fig. 5. *Effect of spermidine, actinomycin D and cycloheximide on the activity of adenosylmethionine decarboxylase in Ehrlich ascites cells treated with difluoromethylornithine*

The cells (initial density $0.11 \times 10^6/\text{ml}$) were grown in the presence of 6 mM-difluoromethylornithine for 24 h. Adenosylmethionine decarboxylase activity was assayed after an exposure of the cells to spermidine ($10 \mu\text{M}$, $\bullet$; $t_{1/2} = 153 \text{ min}$), actinomycin D ($2 \mu\text{g}/\text{ml}$, $\square$; $t_{1/2} = 161 \text{ min}$) or cycloheximide ($10 \mu\text{g}/\text{ml}$, $\blacktriangle$; $t_{1/2} = 62 \text{ min}$) for 0–4 h. Enzyme activities are given relative to the activity at 0 h. For the fit of the lines, see the legend for Fig. 3.

as putrescine and its unsaturated derivative diaminobutene slightly decreased the enhanced adenosylmethionine decarboxylase activity, diamines with longer carbon chains had virtually no effect at all (Table 2). Obviously, regulation of adenosylmethionine decarboxylase was a property of higher polyamines alone, since more than two amino groups were apparently required for the effect. As shown in Table 2, 3,3'-iminodipropylamine was equally potent in repressing difluoromethylornithine-stimulated adenosylmethionine decarboxylase activity as the natural polyamines.

Effect of spermidine, actinomycin D and cycloheximide on adenosylmethionine decarboxylase stimulated by difluoromethylornithine

The possible site of regulation of adenosylmethionine decarboxylase by polyamines was investigated by comparing the effect of spermidine with that of actinomycin D, an inhibitor of RNA synthesis, and cycloheximide, a general inhibitor of protein synthesis. As illustrated in Fig. 5, spermidine and actinomycin D, when added to cultures treated with difluoromethylornithine for 24 h, caused a strikingly parallel decay of adenosylmethionine decarboxylase activity, with an apparent half-life of 153 min and 161 min respectively. Inhibition of protein synthesis by cycloheximide resulted in a swifter disappearance of the enzyme activity, with a half-life of 62 min (Fig. 5). In addition, actinomycin D, when added shortly after the dilution of high-density cell culture, totally blocked induction of adenosylmethionine decarboxylase by difluoromethylornithine, as did also spermidine (results not shown). These results tend to suggest that regulation of adenosylmethionine decarboxylase by polyamines may take place at the transcriptional level of gene expression.

The fact that the half-life of adenosylmethionine

In order to elucidate the specificity of the amine repression of adenosylmethionine decarboxylase, various diamines and polyamines were tested for their ability to block the difluoromethylornithine-induced stimulation of the enzyme activity. Where-

decarboxylase in cells exposed to difluoromethylornithine in this experiment (Fig. 5) was shorter than that shown in Fig. 3 is most probably related to the more pronounced stimulation of the enzyme in the latter experiment. As mentioned above, some dependence was found between the enzyme activities and the corresponding half-lives, the ratio of these variables being proportional to the rate of enzyme synthesis.

Discussion

Complete intracellular deprivation of polyamines has appeared to be a difficult task to achieve. Among the currently available inhibitors of polyamine synthesis, difluoromethylornithine obviously is one of the most useful because of its specificity and potency of inhibition of ornithine decarboxylase (Metcalf *et al.*, 1978). It efficiently depletes cells of putrescine and spermidine. However, inhibition of ornithine decarboxylase by difluoromethylornithine may not be complete, resulting, in combination with the stimulation of adenosylmethionine decarboxylase, in maintenance of normal spermine accumulation. The stimulation of adenosylmethionine decarboxylase is not specific to difluoromethylornithine, since the same effect, although less pronounced, was seen in Ehrlich ascites cells treated with diamino propane and diaminopropanol.

In the present study, inhibitors of ornithine decarboxylase produced a pronounced polyamine deficiency, which was accompanied by a striking time-dependent increase in adenosylmethionine decarboxylase activity. The observation that spermidine and spermine readily repressed the stimulated enzyme activity likewise supports the view that adenosylmethionine decarboxylase is regulated by polyamines. Neither unphysiological diamines nor probably putrescine itself could exert the same effect. The delay in the repression of the enzyme activity obtained with putrescine possibly reflects the time required for the synthesis of spermidine and spermine from the diamine.

In the light of the present results, stimulation of adenosylmethionine decarboxylase appears to involve two mechanisms: (1) a stabilization of the enzyme against degradation and/or (2) an apparent induction of increased synthesis of enzyme protein (as revealed from the kinetic data). Both processes seem to be regulated by polyamines. The suggested mechanism of regulation of enzyme synthesis is that polyamines prevent enhanced gene activation rather than enhanced translation of the enzyme protein on pre-existing mRNA. This situation closely resembles that of regulation of ornithine decarboxylase by diamines in the very early periods of liver regeneration (Kallio *et al.*, 1977), when synthesis of mRNA for ornithine decarboxylase is needed

(Fausto, 1971; Russell & Snyder, 1969). Irrespective of the exact site of regulation, the marked stimulation of adenosylmethionine decarboxylase, as a consequence of even partial polyamine deficiency brought about by inhibitors of ornithine decarboxylase, renders the achievement of total polyamine depletion very difficult.

Regulation of ornithine decarboxylase has been extensively studied both in cultured cells and *in vivo* (for references see Jänne *et al.*, 1978), and several lines of evidence support the central role of putrescine in the regulation of polyamine synthesis. Adenosylmethionine decarboxylase has not received as much attention as ornithine decarboxylase, although its importance in the synthesis of higher polyamines is obvious. In a number of eukaryotic systems stimulated to proliferate, accumulation of putrescine, an activator of adenosylmethionine decarboxylase, appears to be responsible for increases in the enzyme activity (Pegg & Williams-Ashman, 1969; Morris & Fillingame, 1974; Raina & Jänne, 1975; Tabor & Tabor, 1976). In such situations adenosylmethionine decarboxylase may be the rate-limiting enzyme in the synthesis of spermidine. Enhanced enzyme activities have, however, also been observed when determinations *in vitro* were performed in the presence of saturating concentrations of putrescine, which suggests involvement of increased amounts of enzyme protein. Observations that adenosylmethionine decarboxylase was regulated by increases in the enzyme protein during liver regeneration (Hannonen *et al.*, 1972) and in rat prostate stimulated by androgens (Pegg *et al.*, 1970) have been confirmed by direct measurement of the amount of enzyme protein by immunotitration (Pegg, 1979).

Accumulation of active adenosylmethionine decarboxylase in cells exposed to methylglyoxal bis(guanyldrazone), a competitive inhibitor of the enzyme, has been shown to result from a striking stabilization caused by the binding of the inhibitor to the enzyme (Pegg *et al.*, 1973; Fillingame & Morris, 1973; Pegg & Jefferson, 1974; Pegg, 1979). As shown in the present paper, the situation is quite different when adenosylmethionine decarboxylase is stimulated by inhibitors of ornithine decarboxylase. These inhibitors are not supposed to have any affinity for adenosylmethionine decarboxylase, suggesting that some other mechanism(s) than a direct stabilization must be involved. Although the exact molecular mechanisms of the regulation of adenosylmethionine decarboxylase remain to be determined, it is tempting to speculate that higher polyamines exert a repression-like effect on the transcription of the enzyme gene.

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