Biochem. J. (1989) 257, 929–931 (Printed in Great Britain)

Feedback regulation of *S*-adenosylmethionine decarboxylase synthesis

Lo PERSSON,*§ Alex R. KHOMUTOV† and Radii M. KHOMUTOV‡

*Department of Physiology, University of Lund, S-223 62 Lund, Sweden, †N. D. Zelinsky Institute of Organic Chemistry, Academy of Sciences of U.S.S.R., 117913 Moscow B-334, U.S.S.R., and ‡Institute of Molecular Biology, Academy of Sciences of U.S.S.R., 117984 Moscow B-334, U.S.S.R.

Treatment of Ehrlich ascites-tumour cells with 1-amino-oxy-3-aminopropane (AOAP), a potent inhibitor of ornithine decarboxylase, resulted in a marked decrease in cellular contents of putrescine and spermidine, concomitant with an arrest of cell growth. The activity of *S*-adenosylmethionine decarboxylase (AdoMetDC) was greatly increased in cells treated with AOAP. This increase in AdoMetDC activity was shown to be, at least partly, caused by enhanced synthesis of the enzyme, which most likely was induced by the change in cellular polyamine content.

INTRODUCTION

Cellular growth is associated with an increase in the biosynthesis of the polyamines putrescine, spermidine and spermine [1–3]. In fact, this biosynthesis is a prerequisite for the continuation of the growth. Hence the polyamine-synthesis pathway is a potential target for therapeutic agents against various proliferative disorders [4,5]. The rate-limiting steps in polyamine synthesis are catalysed by ornithine decarboxylase (ODC) and *S*-adenosylmethionine decarboxylase (AdoMetDC). These enzymes appear to be subjected to a strict feedback control by the polyamines [3]. Interference with cellular polyamine concentrations usually results in a compensatory change in the activity of one or both of these enzymes. The underlying mechanism(s), however, is not yet fully understood.

In the present work we have used 1-amino-oxy-3-aminopropane (AOAP), which is a potent inhibitor of ODC [6,7], to study the feedback regulation of ODC and AdoMetDC in Ehrlich ascites-tumour cells. Treatment with AOAP resulted in a decrease in cellular putrescine and spermidine contents concomitant with a retardation of cell proliferation. ODC activity was undetectable, whereas the activity of AdoMetDC was markedly increased in cells grown in the presence of AOAP. Analysis of [^{35}S]methionine incorporation revealed a dramatic increase in the synthesis of both ODC and AdoMetDC after treatment with the inhibitor.

EXPERIMENTAL

Materials

AOAP was synthesized as described in [8]. [^{35}S]-Methionine (> 1000 Ci/mmol) was obtained from Amersham Corp. L-[1-^{14}C]Ornithine (54 Ci/mol) and *S*-adenosyl[*carboxy*-^{14}C]methionine (60 Ci/mol) were purchased from New England Nuclear. Antibodies against AdoMetDC were raised in rabbits by using homogeneous preparations of the rat prostate enzyme [9].

Cell culture

Ehrlich ascites-tumour cells were grown in a 1:1 (v/v) mixture of Eagle's minimum essential medium and Ham's F12 medium, supplemented with 0.2% bovine serum albumin and antibiotics. Cells were seeded at a density of 1.0×10^5 cells/ml in the absence or presence of 1.0 mM-AOAP.

Analysis of ODC and AdoMetDC activities

Cells were sonicated in ice-cold 0.1 M-Tris/HCl, pH 7.5, containing 0.1 mM-EDTA and 0.5 mM-dithiothreitol. After centrifugation at 20000 *g* for 20 min, ODC and AdoMetDC activities were assayed essentially as described in [10] and [11] respectively.

Analysis of ODC and AdMetDC syntheses

Syntheses of ODC and AdoMetDC were determined by measuring the incorporation of [^{35}S]methionine into the enzymes. The cells were reseeded (1.0×10^6 cells/ml) in methionine-free medium. After 10 min of preincubation at 37 °C, the cultures were supplemented with [^{35}S]methionine (10 μCi/ml) and incubated for an additional 25 min. The cells were then sonicated as described above. Samples of the supernatants (containing equal amounts of acid-insoluble radioactivity) were incubated with an excess of either anti-ODC antiserum [12] or anti-AdoMetDC antiserum at room temperature for 30 min. Enzyme bound to the antibody was precipitated, washed and fractionated by SDS/PAGE essentially as described in [13].

Analysis of polyamine content

The cellular polyamine content was determined by using an automatic amino acid analyser (Biotronik LC 5001) with *o*-phthaldialdehyde as reagent.

RESULTS AND DISCUSSION

AOAP is a potent inhibitor of ODC with effects at as low as nanomolar concentrations [6]. At physiological

Abbreviations used: AdoMetDC, *S*-adenosylmethionine decarboxylase (EC 4.1.1.50); AOAP, 1-amino-oxy-3-aminopropane; ODC, ornithine decarboxylase (EC 4.1.1.17); PAGE, polyacrylamide-gel electrophoresis.

§ To whom correspondence and reprint requests should be addressed.

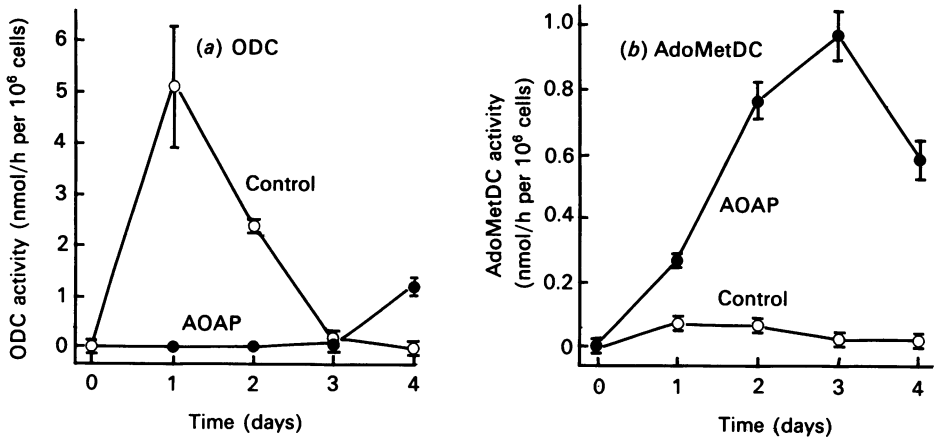

Fig. 1. Effects of AOAP on ODC (*a*) and AdoMetDC (*b*) activities

Cells were grown in the absence (○) or presence (●) of 1.0 mM-AOAP. Values are means ± S.E.M. of three determinations.

Table 1. Effects of AOP on cell growth and polyamine content

Cells were seeded in the absence or presence of 1.0 mM-AOAP. Values are means ± S.E.M. of three determinations.

Treatment	Day	10^{-5} × Cell no./ml	Polyamine content (nmol/10^6 cells) Putrescine	Spermidine	Spermine
Control	0	1.0±0	0.1±0.02	1.4±0.04	1.0±0.03
Control	1	1.6±0.1	1.0±0.20	2.5±0.38	1.1±0.03
AOAP	1	1.6±0.1	<0.1	0.3±0.04	1.4±0.15
Control	2	5.3±0.1	1.1±0.06	3.0±0.19	1.1±0.08
AOAP	2	3.7±0.2	<0.1	0.1±0.04	1.2±0.22
Control	3	17.0±1.4	0.4±0.06	1.9±0.24	1.1±0.13
AOAP	3	5.5±0.3	<0.1	0.2±0.12	0.9±0.14
Control	4	21.6±1.3	0.7±0.08	1.8±0.15	1.0±0.06
AOAP	4	8.6±0.4	<0.1	0.4±0.03	1.8±0.16

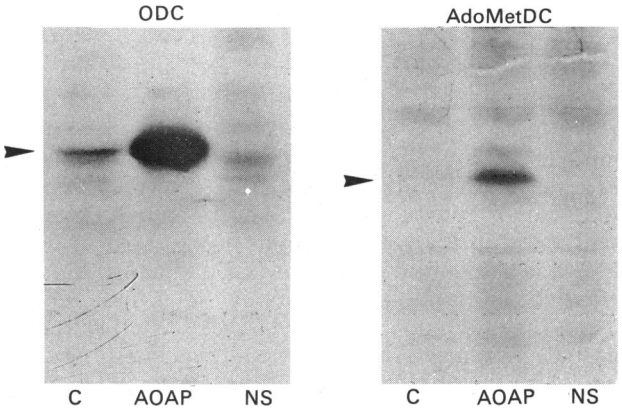

Fig. 2. Effects of AOAP on syntheses of ODC (*a*) and AdoMetDC (*b*)

Cells were grown in the absence or presence of 1.0 mM-AOAP for 2 days. Lanes: C, control cells; AOAP, cells grown in the presence of AOAP; NS, non-specific binding of antibodies. Arrows indicate the migration of radiolabelled ODC (M_r = 33000).

pH AOAP exists as a monocation (the pK of the H_2NO group is 4.5–5.0). The positively charged amino group probably plays an anchoring function in the enzyme–inhibitor complex, and is thus important for the proper orientation of AOAP in the active centre. The specificity of AOAP is confirmed by the fact that a more than 10000-fold higher concentration of the inhibitor has to be used to inhibit ornithine transaminase or AdoMetDC [7].

When Ehrlich ascites-tumour cells were seeded in fresh medium, a marked induction of ODC and AdoMetDC activities occurred during the exponential growth of the cells (Fig. 1). The increases were accompanied by elevated contents of putrescine and spermidine (Table 1). When added to the medium, AOAP (1.0 mM) eradicated the increase in ODC activity. Thus ODC activity remained undetectable during the first 2 days in the presence of AOAP, whereafter a small increase was observed (Fig. 1). The inhibition of ODC activity by AOAP was reflected in decreases in the cellular contents of putrescine and spermidine, whereas the spermine content remained virtually unchanged (Table 1).

As seen in Table 1, the growth of the cells was also affected by AOAP. Cells seeded in the presence of AOAP grew significantly slower than those grown without AOAP.

In spite of the slight inhibitory effect of AOAP on AdoMetDC *in vitro*, a dramatic increase was observed in cells exposed to AOAP (Fig. 1). AdoMetDC activity increased more than 100-fold when cells were seeded in a medium containing AOAP. Without AOAP the growth-induced increase in AdoMetDC activity was about 10-fold.

The polyamines have been shown to exert a feedback control of ODC synthesis. A rise in cellular polyamine content results in a decrease in ODC synthesis [14–17], whereas a decrease in polyamine content gives rise to an increased ODC synthesis [15,18]. These polyamine-mediated changes in ODC synthesis appear to be carried out exclusively at the translational level, since no changes are seen in the amount of ODC mRNA [14–18].

The knowledge of AdoMetDC regulation, on the other hand, is more sparse. Treatment of cells with various inhibitors of polyamine synthesis is often accompanied by an increase in AdoMetDC activity [19–21]. The mechanism behind this increase has not yet been

fully understood. Part of the increase can sometimes be explained by a decreased turnover of the enzyme [19–21]. However, the major part of the increase can only be explained by either an activation of pre-existing non-active enzyme molecules or an increased synthesis of the enzyme. Pegg and co-workers [22,23] have demonstrated that the amount of translatable AdoMetDC mRNA, as measured in a translation system *in vitro*, is increased after inhibition of polyamine synthesis, indicating an effect of AdoMetDC synthesis.

In order to investigate whether the AOAP-induced increase in AdoMetDC activity was due to a rise in the synthesis of the enzyme, we pulse-labelled cells grown with or without AOAP for 2 days. The incorporation of radioactivity into AdoMetDC and ODC was then determined by immunoprecipitation and gel electrophoresis. The autoradiograms are shown in Fig. 2, and show that the synthesis of AdoMetDC, as well as that of ODC, was greatly stimulated in cells treated with AOAP. The observed change in synthesis rate was most likely related to the changes in cellular polyamine concentrations caused by AOAP. Hence it appears that the polyamines exert a feedback control not only of the synthesis of ODC but also of that of AdoMetDC.

This study was supported by grants from the Swedish Medical Research Council (04X-02212), the Medical Faculty (University of Lund), and The Magnus Bergvall, John and Augusta Persson, and Lars Hierta's Memory Foundations.

REFERENCES

1, Heby, O. (1981) Differentiation **20**, 1–19
2. Tabor, C. W. & Tabor, H. (1984) Annu. Rev. Biochem. **53**, 749–790
3. Pegg, A. E. (1986) Biochem. J. **234**, 249–262
4. McCann, P. P., Pegg, A. E. & Sjoerdsma, A. (1987) Inhibition of Polyamine Metabolism, Academic Press, Orlando
5. Pegg, A. E. (1988) Cancer Res. **48**, 759–774
6. Khomutov, R. M., Denisova, G. F., Khomutov, A. R., Belostotskaya, K. M., Shlosman, R. B. & Artamonova, E. Y. (1985) Bioorg. Khim. **11**, 1574–1576
7. Khomutov, A. R., Gabibov, A. G., Khurs, E. N., Tolosa, E. A., Shuster, A. M., Goryachenkova, E. V. & Khomutov, R. M. (1987) in Biochemistry of Vitamin B_6 (Korpela, T. & Christen, P., eds.), pp. 317–320, Birkahäuser Verlag, Basel
8. Khomutov, A. R. (1988) Bioorg. Khim., in the press
9. Pösö, H. & Pegg, A. E. (1982) Biochemistry **21**, 3116–3122
10. Jänne, J. & Williams-Ashman, H. G. (1971) Biochem. Biophys. Res. Commun. **42**, 222–229
11. Pegg, A. E. & Williams-Ashman, H. G. (1969) J. Biol. Chem. **244**, 682–693
12. Persson, L. (1982) Acta Chem. Scand. **B36**, 685–688
13. Persson, L., Seely, J. E. & Pegg, A. E. (1984) Biochemistry **23**, 3777–3783
14. Kahana, C. & Nathans, D. (1985) J. Biol. Chem. **260**, 15390–15393
15. Persson, L., Holm, I. & Heby, O. (1986) FEBS Lett. **205**, 175–178
16. Hölttä, E. & Pohjanpelto, P. (1986) J. Biol. Chem. **261**, 9502–9508
17. Persson, L., Holm, I. & Heby, O. (1988) J. Biol. Chem. **263**, 3528–3533
18. Persson, L., Oredsson, S. M., Anehus, S. & Heby, O. (1985) Biochem. Biophys. Res. Commun. **131**, 239–245
19. Alhonen-Hongisto, L. (1980) Biochem. J. **190**, 747–754
20. Mamont, P. S., Joder-Olenbusch, A.-M., Nussli, M. & Grove, J. (1981) Biochem. J. **196**, 411–422
21. Shirahata, A. & Pegg, A. E. (1985) J. Biol. Chem. **260**, 9583–9588
22. Shirahata, A. & Pegg, A. E. (1986) J. Biol. Chem. **261**, 13833–13837
23. Pegg, A. E., Wechter, R. & Pajunen, A. (1987) Biochem. J. **244**, 49–54

Received 24 October 1988; received 1 December 1988