Gene expression of ornithine decarboxylase in L1210 leukaemia cells exposed to DL-2-difluoromethylornithine in the presence of cadaverine

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Cultured mouse L1210 leukaemia cells treated with DL-2-difluoromethylornithine, an irreversible inhibitor of ornithine decarboxylase (EC 4.1.1.17), in the presence of micromolar concentrations of cadaverine, started to overproduce ornithine decarboxylase after an exposure of several weeks. The more than 60-fold excess of the enzyme protein in the drug-treated cells apparently resulted from a strikingly enhanced accumulation of mRNA for the enzyme associated with only a modest (about 2-fold) gene amplification.

INTRODUCTION

ODC is one of about ten mammalian enzymes the genes for which have been demonstrated to undergo amplification under appropriate selection pressure (Schimke, 1984). Coffino and co-workers (McConlogue & Coffino, 1983; McConlogue *et al.*, 1984) selected a mouse lymphoma cell line resistant to DFMO, which resulted from gene-amplification-based overproduction of the enzyme. Similarly, Kahana & Nathans (1984) selected a mouse myeloma cell line grown under the pressure of DFMO in which the ODC gene(s) were amplified. We recently selected an Ehrlich ascites-carcinoma cell line exposed to stepwise-increasing concentrations of DFMO, which overproduced ODC owing to a 10–20-fold increase in the gene dosage of the enzyme (Alhonen-Hongisto *et al.*, 1985).

In a series of experiments aimed to elucidate the specificity of polyamine requirement for cell proliferation, we grew Ehrlich ascites-carcinoma cells in the presence of DFMO and cadaverine (Alhonen-Hongisto *et al.*, 1982). This treatment led to an almost complete replacement of the natural polyamines (putrescine, spermidine and spermine) by cadaverine-based [aminopropylcadaverine and bis(aminopropyl)cadaverine] polyamines (Alhonen-Hongisto *et al.*, 1982). Although the new polyamine pattern was compatible with slow growth, these cells behaved exactly like polyamine-depleted cells, with strikingly elevated *S*-adenosylmethionine decarboxylase activity and stimulation of the putative polyamine carrier (Alhonen-Hongisto *et al.*, 1982). In spite of the continuous presence of DFMO, these cells also exhibited dramatically enhanced ODC activity (Alhonen-Hongisto *et al.*, 1982).

We have now exposed L1210 mouse leukaemia cells to DFMO in the presence of cadaverine in order to study whether the observed enhancement of ODC activity is based on an enhanced gene expression leading to an overproduction of the enzyme protein.

EXPERIMENTAL

Cell cultures

Mouse L1210 leukaemia cells were cultured in RPMI 1640 medium supplemented with 5% (v/v) pooled human serum (Finnish Red Cross Transfusion Service, Helsinki, Finland) and antibiotics. The cells were first exposed to 5 mM-DFMO in the presence of 5 μ M-cadaverine for 10 weeks, whereafter the concentration of DFMO was increased to 10 mM without changing the concentration of cadaverine.

Materials

DFMO was generously given by Centre de Recherche Merrell International (Strasbourg, France). [5-³H]DFMO (sp. radioactivity 26.5 Ci/mmol) was purchased from New England Nuclear Corp., Dreieich, West Germany. DL-[1-¹⁴C]Ornithine (sp. radioactivity 57 Ci/mol) and 5'-[α -³²P]dCTP (sp. radioactivity 410 Ci/mmol) were purchased from Amersham International (Amersham, Bucks., U.K.). For hybridization studies, a cDNA clone (pODC16) encoding mouse kidney ODC (Kontula *et al.*, 1984; Jänne *et al.*, 1984) was used.

Analytical methods

Polyamines were determined by the method of Seiler (1970), by using the solvent system of Hölttä *et al.* (1979). ODC activity was assayed by the method of Jänne & Williams-Ashman (1971). Immunoreactive ODC was determined by the method of Erwin *et al.* (1983), by using monospecific antibody raised in rabbits (Isomaa *et al.*, 1983). Genomic DNA was isolated by the method of Blin & Stafford (1976). Isolated DNA was digested with *Eco*RI restriction endonuclease, electrophoresed in 0.9% agarose gels, transferred on to nitrocellulose filters (Southern, 1975) and hybridized to nick-translated (Rigby *et al.*, 1977) pODC16. Total cellular RNA was isolated by the method of Auffray & Rougeon (1980). RNA was fractionated by electrophoresis in 1.4%

Abbreviations used: ODC, ornithine decarboxylase (EC 4.1.1.17); DFMO, DL-2-difluoromethylornithine.

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Table 1. Polyamine concentrations (fmol/cell) in untreated tumour cells and in cells exposed to DFMO in the presence of cadaverine

Abbreviation: n.d., not detected.

Treatment	Putrescine	Cadaverine	Spermidine	Aminopropylcadaverine	Spermine	Bis(aminopropyl)cadaverine
None	0.98	n.d.	4.85	n.d.	0.88	n.d.
DFMO + cadaverine	n.d.	2.00	0.09	1.88	0.70	0.04

agarose gels in the presence of 1 M-glyoxal, transferred on to Gene Screen membranes (New England Nuclear Corp.) and hybridized to nick-translated pODC16 (Thomas, 1980). Dot-blot analyses were performed as described by Thomas (1980) by using a Hybri-Dot Manifold from Bethesda Research Laboratories, Gaithersburg, MD, U.S.A.

RESULTS

L1210 leukaemia cells grew readily in culture in the presence of DFMO and cadaverine, but failed to grow in the presence of DFMO alone. The intracellular polyamine pattern of untreated tumour cells and cells exposed to DFMO in the presence of cadaverine for about 10 weeks is shown in Table 1. The treatment resulted in a replacement of putrescine and spermidine by cadaverine and aminopropylcadaverine, yet the content of spermine remained almost unchanged. The total polyamines (both the natural and the cadaverine-based) in the drug-treated cells amounted to more than 80% of that found in the untreated cells (Table 1). In spite of the near-normal amine content and of the presence of high (10 mM) DFMO concentration, dialysed extracts of the 'cadaverine cells' exhibited about twice as much ODC activity and contained 60 times as much immunoreactive ODC protein as the untreated cells (Table 2). The discrepancy between the enhancement of enzyme activity and the amount of enzyme protein in the drug-treated cells is obviously attributable to the fact that enzyme inactivated by DFMO also reacts with the antibody.

The striking overproduction of the enzyme was obviously based on a dramatic stimulation of the accumulation of the enzyme's mRNA, as illustrated by the hybridization analyses in Fig. 1. As also shown, the enhanced expression of ODC was associated with an increase in the gene dosage of the enzyme, as sequences residing in a 6.6-kilobase *Eco*RI fragment were clearly amplified in the drug-treated cells (Fig. 1). As revealed from the semi-quantitative dot-blot analyses (Fig. 2), the increase in the gene dosage was not more than 2-fold, in

Fig. 1. Restriction analysis (*Eco*RI) of ornithine decarboxylase sequences in genomic DNA (a) and amounts of ornithine decarboxylase mRNA (b)

DNA and RNA were isolated from untreated L1210 leukaemia cells (Co) or from cells exposed to DFMO and cadaverine for about 2 months (DFMO + Cad). Abbreviation: kb, kilobases.

Table 2. Ornithine decarboxylase activity and the amount of immunoreactive protein in untreated tumour cells and in cells exposed to DFMO in the presence of cadaverine

Treatment	ODC activity (nmol/30 min per 10 ⁶ cells)	ODC protein (ng/10 ⁶ cells)
None	0.50	0.70
DFMO + cadaverine	0.93	44

Fig. 2. Dot-blot analyses of ornithine decarboxylase sequences in genomic DNA (a) and amounts of mRNA for the enzyme (b)

The amount of nucleic acids applied is indicated. Column 1, untreated cells; column 2, cells exposed to DFMO and cadaverine.

comparison with the 20–30-fold increase in the hybridizable RNA sequences isolated from the cells exposed to DFMO and cadaverine. The process of gene amplification was relatively slow, as the first signs of increased gene dosage were seen not earlier than after 2 months' continuous exposure to the drugs, whereas the increased accumulation of mRNA was evident already after 1 month in culture (results not shown).

DISCUSSION

It is obvious that ODC is one of those enzymes the gene dosage for which easily changes under appropriate selection conditions. In all cases described so far, the extent of gene amplification of the enzyme has been substantial (McConlogue *et al.*, 1984; Kahana & Nathans, 1984; Alhonen-Hongisto *et al.*, 1985), and obviously markedly contributes to the overproduction of the enzyme. Relatively little is known about the regulation of the transcription of ODC. In connection with their initial cloning of cDNA encoding mouse kidney ODC, Kontula *et al.* (1984) showed that androgen administration produced a marked enhancement of mRNA accumulation in mouse kidney. Similarly, Kahana & Nathans (1984) provided evidence indicating that the well-known stimulation of ODC on dilution of cultured cells with fresh medium was associated with an increase in mRNA accumulation. The DFMO-resistant Ehrlich ascites-carcinoma cell line that we (Alhonen-Hongisto *et al.*, 1985) selected contained 20 times more immunoreactive ODC than did the parental cell line. Interestingly, the enhanced expression of the enzyme appeared to be almost totally attributable to the increased gene dosage (10–20-fold) and did not necessarily require any specific stimulation of the transcription.

The overproduction of ODC in L1210 leukaemia cells exposed to DFMO in the presence of cadaverine is clearly based on a strikingly enhanced accumulation of mRNA (20–30-fold), with only marginal changes of the gene dosage. It is noteworthy that the cadaverine-based polyamines apparently do not fulfil the roles of the natural polyamines as regards the regulation of ODC gene expression and other compensatory mechanisms (Alhonen-Hongisto *et al.*, 1982) triggered by polyamine depletion. As the enhanced accumulation of mRNA for ODC appears to precede any increases of gene dosage,

this system could serve as a model for studies directed to the elucidation of the mechanism of gene amplification. Especially interesting is the possible causal relationship between the enhanced transcription and the development of gene amplification, as this may be one of the general mechanisms leading to the amplified genotype (Schimke, 1984).

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REFERENCES

- Alhonen-Hongisto, L., Seppänen, P., Hölttä, E. & Jänne, J. (1982) *Biochem. Biophys. Res. Commun.* **106**, 291–297
- Alhonen-Hongisto, L., Kallio, A., Sinervirta, R., Seppänen, P., Kontula, K. K., Jänne, O. A. & Jänne, J. (1985) *Biochem. Biophys. Res. Commun.* **126**, 734–740
- Auffray, C. & Rougeon, F. (1980) *Eur. J. Biochem.* **107**, 303–314
- Blin, N. & Stafford, D. W. (1976) *Nucleic Acids Res.* **3**, 2303–2308
- Erwin, B. G., Seely, J. E. & Pegg, A. E. (1983) *Biochemistry* **22**, 3027–3032
- Hölttä, E., Jänne, J. & Hovi, T. (1979) *Biochem. J.* **178**, 109–117
- Isomaa, V. V., Pajunen, A. E. I., Bardin, C. W. & Jänne, O. A. (1983) *J. Biol. Chem.* **258**, 6735–6740
- Jänne, J. & Williams-Ashman, H. G. (1971) *J. Biol. Chem.* **246**, 1725–1732
- Jänne, O. A., Kontula, K. K., Isomaa, V. V. & Bardin, C. W. (1984) *Ann. N.Y. Acad. Sci.* **438**, 72–84
- Kahana, C. & Nathans, D. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3645–3649
- Kontula, K. K., Torkkeli, T. K., Bardin, C. W. & Jänne, O. A. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 731–735
- McConlogue, L. & Coffino, P. (1983) *J. Biol. Chem.* **258**, 8384–8388
- McConlogue, L., Gupta, M., Wu, L. & Coffino, P. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 540–544
- Rigby, P. W. J., Dieckman, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–351
- Schimke, R. T. (1984) *Cell* **37**, 705–713
- Seiler, N. (1970) *Methods Biochem. Anal.* **18**, 259–337
- Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517
- Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5201–5205

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