Inhibition of rat heart ornithine decarboxylase by basic polypeptides

Flavio FLAMIGNI, Carlo GUARNIERI,* Sandra MARMIROLI and Claudio M. CALDARERA
Istituto di Chimica Biologica, Università di Bologna, Via Irnerio 48, 40126 Bologna, Italy

(Received 18 February 1985/12 April 1985; accepted 18 April 1985)

Purified and partially purified ornithine decarboxylase (ODC) from rat heart was inhibited by basic polypeptides *in vitro*. Poly-L-arginine, the most effective, was inhibitory at a concentration as low as 0.1 µg/ml; protamine and histone clearly inhibited ODC at concentrations higher than 2 µg/ml, but poly-L-lysine was less effective. The ability to inhibit ODC appeared to correlate with the arginine-residue content of basic polypeptides. The inhibition effect could be decreased by increasing substrate concentration and ionic strength.

The regulation of ODC, which catalyses the first and rate-limiting step in polyamine synthesis, is of particular interest, since ODC activity undergoes a large, rapid and transient increase after hormonal and growth stimuli and has an exceptionally short half-life. In androgen-treated mouse kidney, increased ODC activity is a result of an increase in the amount of enzyme protein, supported by an accumulation of ODC mRNA and a slowed ODC degradation (Persson *et al.*, 1984; Kontula *et al.*, 1984). On the other hand, in liver of thioacetamide-treated rats, a discrepancy was detected between the increase in enzyme amount and enzyme activity (Kameji *et al.*, 1984) and, according to Koenig *et al.* (1983), an extremely early (<2 min) rise in ODC activity, which cannot be accounted for by ODC induction, occurs in a rapid plasma-membrane response to various stimuli. Actually, post-translational modifications of the ODC molecule have been proposed (Russell & Manen, 1982; Kuehn & Atmar, 1983; Meggio *et al.*, 1984), and a non-competitive protein inhibitor, termed ODC-antizyme, can be elicited in a variety of cultured cells as well as in animals (Heller & Canellakis, 1981; Fujita *et al.*, 1984). Besides, mammalian ODC activity has been shown to be affected by salts (Kitani & Fujisawa, 1984), phospholipids (Kitani & Fujisawa, 1981b), phosphoryl-ethanolamine (Gilad & Gilad, 1984) and various polyanionic compounds (Kitani & Fujisawa, 1981a), although often at relatively high concentrations.

Two basic proteins that inhibit ODC have been isolated from *Escherichia coli* (Heller *et al.*, 1983);

one of these has an amino acid composition similar to that of the *E. coli* histone-like protein HU and the eukaryotic histone F2b, whereas the other is characterized by an unusually high arginine content. This finding, and the observation that ODC may be abundant in nuclei (Emanuelsson & Heby, 1982), where it might be implicated in regulation of rRNA synthesis (Russell & Manen, 1982; Kuehn & Atmar, 1983), prompted us to investigate whether mammalian ODC (purified from rat heart) could be inhibited by histones or other basic polypeptides *in vitro*.

Experimental

L-[1-¹⁴C]Ornithine (sp. radioactivity 52.7 Ci/mol) was purchased from New England Nuclear. Poly-L-lysine hydrochloride (170 residues), poly-L-arginine hydrochloride (200 residues), unfractionated histones from calf thymus (type II-AS), histone fractions F1 (type V-S), F2a (type VI-S), F2b (type VII-S) and F3 (type VIII-S), and protamine chloride from salmon sperm (grade V) were purchased from Sigma Chemical Co. The indicated concentrations were not corrected for chloride content. ODC was highly purified from hearts of rats treated with 10 mg of isoprenaline/kg body wt. as previously published (Meggio *et al.*, 1984) by using (NH₄)₂SO₄ fractionation, DE-52 DEAE-cellulose chromatography, Sephadex G-200 gel filtration and pyridoxamine phosphate-agarose affinity chromatography (Flamigni *et al.*, 1984).

ODC activity was measured essentially as described by Heller & Canellakis (1981). Incubation medium contained, in a 0.05 ml final volume: 0.04 M-Tris/HCl (pH 7.2 at 25°C), 0.1 mM-EDTA,

* To whom correspondence and reprint requests should be addressed.

35 μ M-pyridoxal phosphate, 3 mM-dithiothreitol, 0.015% Brij 35, the indicated concentration of L-ornithine and 0.05 unit of ODC (1 unit of enzyme represents the amount releasing 1 nmol of CO₂/min in the presence of 0.45 mM-L-ornithine). The inhibitors were added just before the incubation, which was carried out at 37°C for 30 min in a shaking bath.

Results

The addition of various basic synthetic polypeptides as well as naturally occurring proteins (20 μ g/ml) inhibited highly purified ODC from rat heart (Table 1). When a subsaturating concentration of the substrate (0.04 mM) was used, the inhibition was more evident. In particular, poly-L-arginine and protamine were the most effective (>90% inhibition), whereas poly-L-lysine was only slightly inhibitory (36%). Of the histone fractions, F3 was the most active (76%), whereas the inhibition by F1 was less (36%). At a concentration of ornithine close to the saturating value (0.45 mM), only polyarginine showed a very consistent inhibitory effect (96%), whereas all the other basic polypeptides decreased ODC activity to a lesser degree. The addition of 0.1 M-NaCl to 0.45 mM-ornithine further decreased the inhibitory effect shown by the various polypeptides. However, the effect of salt was more evident for protamine than for histones; besides this, polyarginine was still markedly inhibitory (92%).

At substrate concentrations near K_m , protamine and histones inhibited ODC activity strongly at doses of a few μ g/ml, whereas poly-L-lysine was scarcely effective even at higher concentrations (Fig. 1). On the contrary, poly-L-arginine appeared to be a very potent inhibitor, since over 50%

inhibition was obtained at a concentration as low as 0.1 μ g/ml. However, 0.01 μ g/ml was ineffective (results not shown).

The inhibition by histones was of a mixed type (Fig. 2a): at 20 μ g/ml, V_{max} was decreased to 65% of control, and the apparent K_m was increased 3.2-fold. A similar kinetic pattern was obtained for histone fraction F3, but the pattern was somewhat

Fig. 1. Effect of increasing concentration of basic proteins on highly purified ODC from rat heart. Δ , Poly-L-lysine; $\blacksquare$, histones (unfractionated); $\blacktriangledown$, protamine; $\blacklozenge$, poly-L-arginine. ODC assay was performed at 0.1 mM-L-ornithine. The results are means $\pm$ S.D. ($n = 3$). Control ODC activity was 24.3 ± 0.12 pmol/min.

Table 1. Effect of various basic proteins on rat heart ODC activity

ODC was highly purified from rat heart as described in the Experimental section. ODC activity is expressed as per cent of control ($\pm$ S.D.; $n = 3$); control activity was 11.2 pmol/min in the presence of 0.04 mM-ornithine, 51.1 pmol/min in the presence of 0.45 mM-ornithine and 37.7 pmol/min in the presence of 0.45 mM-ornithine plus 0.1 M-NaCl. Polypeptide concentration was 20 μ g/ml.

Addition	ODC activity (% of control)		
	Ornithine (0.04 mM)	Ornithine (0.45 mM)	Ornithine (0.45 mM) + NaCl (0.1 M)
Control	100 $\pm$ 0.5	100 $\pm$ 0.9	100 $\pm$ 3.4
Poly-L-lysine	64 $\pm$ 0.5	79 $\pm$ 0.8	100 $\pm$ 1.4
Poly-L-arginine	3 $\pm$ 0.5	4 $\pm$ 0.3	8 $\pm$ 0.2
Protamine	6 $\pm$ 0.2	34 $\pm$ 5.5	84 $\pm$ 2.0
Histone (unfractionated)	16 $\pm$ 0.3	52 $\pm$ 0.1	78 $\pm$ 3.7
Histone fraction:			
F1	64 $\pm$ 0.1	88 $\pm$ 1.3	—
F2a	44 $\pm$ 1.2	76 $\pm$ 0.7	—
F2b	60 $\pm$ 1.5	87 $\pm$ 1.3	—
F3	24 $\pm$ 0.3	59 $\pm$ 1.4	77 $\pm$ 0.5

Fig. 2. Lineweaver-Burk plots of ODC activity as a function of L-ornithine concentration in the presence of various basic proteins

ODC was highly purified from rat heart as described in the Experimental section and then assayed at different L-ornithine concentrations. (a) $\blacktriangle$, control; $\blacksquare$, histones (unfractionated), 10 μ g/ml; $\bullet$, histones, 20 μ g/ml. (b) $\blacktriangle$, Control; $\blacktriangledown$, protamine, 20 μ g/ml; $\blacklozenge$, poly-L-arginine, 0.1 μ g/ml.

different for protamine and poly-L-arginine (Fig. 2b), with a marked increase in K_m (approx. 6-fold by 20 μ g of protamine/ml and approx. 3-fold by 0.1 μ g poly-L-arginine/ml) and with only a slight decrease in V_{max} , if any. The inhibition by basic proteins was also observed with partially purified enzyme (specific activity about 5 nmol/min per mg) and appeared essentially time-independent (results not shown).

Discussion

The present study shows that mammalian ODC may be inhibited by low concentrations of basic

polypeptides, much lower than with polyanions (Kitani & Fujisawa, 1981a): protamine and histones were clearly inhibitory at a few μ g/ml and polyarginine at 0.1 μ g/ml, corresponding to about 2.5 nM. Basic amino acids and polyamines are known to be very weak ODC inhibitors (Pegg & McGill, 1979): K_i values for putrescine, spermidine and lysine were reported to be 1.4 mM, 5.5 mM and 8.8 mM respectively, corresponding to concentrations around 1 mg/ml, whereas 20 mM-arginine was not inhibitory. The inhibition by basic polypeptides was partially prevented by increasing ionic strength, suggesting that interactions between oppositely charged groups may be involved; however, even in this condition (and at high ornithine concentration) polyarginine and histones were still inhibitory. Besides, it may be noted that polylysine was poorly effective, and among histone fractions, F3, which has a basic/acidic residues ratio of 1.8 (Hnilica, 1975) was more inhibitory than F1, which has a basic/acidic ratio of 5.6. The ability to inhibit ODC (polyarginine > protamine > F3 > F2a > F2b > F1) seemed to correlate with the arginine content (Hnilica, 1975), rather than with the net positive charge or the total number of basic residues.

The differences between histones and protamine in the inhibition kinetic patterns and the effect of increased ionic strength suggest that the mechanism(s) of ODC inhibition may be at least partially different for the various proteins.

Unfractionated histones, probably in consequence of a co-operative effect, appear to be more inhibitory than any individual fraction tested.

In the light of the present work, it is noteworthy that, of the two basic proteins inhibiting ODC in *E. coli* (Heller *et al.*, 1983), one of them resembles histone F2b, and the other is particularly rich in arginine residues. Pangiotidis & Canellakis (1984) have identified these two basic proteins as the ribosomal proteins S20/L26 and L34 respectively, suggesting that ODC-ribosome interactions may play a role in regulation of polyamine biosynthesis. Furthermore, the ODC inhibition was relieved by DNA (Huang *et al.*, 1984). *In vitro*, histones and synthetic polycations, such as polylysine or polyarginine, have been found to facilitate nucleosome assembly (Bogdanova, 1984), to activate type-II casein kinase (Meggio *et al.*, 1983), to inhibit Ca^{2+} -dependent protein kinases (Qi *et al.*, 1983), and to modulate protein phosphatases (Schlender & Mellgren, 1984; Di Salvo *et al.*, 1984). Russell *et al.* (1983) have reported the isolation of a polypeptide from hepatoma cells, able to inhibit calf liver ODC and also to prevent the polyamine stimulation of a liver protein kinase.

ODC was demonstrated to be abundant in nuclei by labelling with difluoro[3H]methylornithine

(Emanuelsson & Heby, 1982) and by direct assay (Bartholeyns, 1983). In particular, Bartholeyns (1983) found an increased recovery of ODC in the subcellular fractions compared with the original homogenate, and, in nuclei, he could clearly segregate ODC from an inhibiting, non-diffusable factor. Eukaryotic ODC, as such or after post-translational modifications, has also been regarded as an acidic protein able to control rRNA-gene transcription (Russell & Manen, 1982; Kuehn & Atmar, 1983). From all these observations, the question of a possible inhibition *in vivo* of mammalian ODC, by histones or other basic polypeptides that might occur in cells, arises, and could be worthy of further investigation in view of the supposed roles of ODC and histones in gene expression and growth processes.

We thank Mr. G. Piazza and Mr. S. Rizzuto for their excellent technical assistance and Ms. A. Zarri for her careful secretarial support. This study was supported by a grant from Ministero Pubblica Istruzione, Italy.

References

- Bartholeyns, J. (1983) *Life Sci.* **32**, 1305–1312
- Bogdanova, E. S. (1984) *FEBS Lett.* **175**, 321–324
- Di Salvo, J., Gifford, D. & Kokkinakis, A. (1984) *Proc. Soc. Exp. Biol. Med.* **177**, 24–32
- Emanuelsson, H. & Heby, O. (1982) *Cell Biol. Int. Rep.* **6**, 951–954
- Flamigni, F., Guarnieri, C. & Caldarera, C. M. (1984) *Biochim. Biophys. Acta* **802**, 245–252
- Fujita, K., Matsufuji, S., Murakami, Y. & Hayashi, S. (1984) *Biochem. J.* **218**, 557–562
- Gilad, G. M. & Gilad, V. H. (1984) *Biochem. Biophys. Res. Commun.* **122**, 277–282
- Heller, J. S. & Canellakis, E. S. (1981) *J. Cell. Physiol.* **107**, 209–217
- Heller, J. S., Rostomily, R., Kyriakidis, D. A. & Canellakis, E. S. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 5181–5184
- Hnilica, L. S. (1975) *Methods Enzymol.* **40**, 102–138
- Huang, S., Kyriakidis, D. A., Rinehart, C. A., Jr. & Canellakis, E. S. (1984) *Biochem. Pharmacol.* **33**, 1383–1386
- Kameji, T., Fujita, K., Noguchi, T., Takiguchi, M., Mori, M., Tatibana, M. & Hayashi, S. (1984) *Eur. J. Biochem.* **144**, 35–39
- Kitani, T. & Fujisawa, H. (1981a) *FEBS Lett.* **125**, 83–85
- Kitani, T. & Fujisawa, H. (1981b) *FEBS Lett.* **132**, 296–298
- Kitani, T. & Fujisawa, H. (1984) *Biochim. Biophys. Acta* **784**, 164–167
- Koenig, H., Goldstone, A. D. & Lu, C. Y. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 7210–7214
- Kontula, K. K., Torkeli, T. K., Bardin, C. W. & Jänne, O. A. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 731–735
- Kuehn, G. D. & Atmar, V. J. (1983) *Adv. Polyamine Res.* **4**, 615–629
- Meggio, F., Brunati, A. M. & Pinna, L. A. (1983) *FEBS Lett.* **160**, 203–208
- Meggio, F., Flamigni, F., Caldarera, C. M., Guarnieri, C. & Pinna, L. A. (1984) *Biochem. Biophys. Res. Commun.* **122**, 997–1004
- Pangiotidis, C. A. & Canellakis, E. S. (1984) *J. Biol. Chem.* **259**, 15025–15027
- Pegg, A. E. & McGill, S. (1979) *Biochim. Biophys. Acta* **568**, 416–427
- Persson, L., Seely, J. E. & Pegg, A. E. (1984) *Biochemistry* **23**, 3777–3783
- Qi, D., Schatzman, R. C., Mazzei, G. I., Turner, R. S., Raynor, R. L., Liao, S. & Kuo, J. F. (1983) *Biochem. J.* **213**, 281–288
- Russell, D. H. & Manen, C. A. (1982) *Biochem. Pharmacol.* **31**, 3373–3378
- Russell, D. H., Criss, W. E., Oka, T., Marishita, Y., Akogyeram, C. & Barinder, D. (1983) *J. Nutr. Growth Cancer* **1**, 91–95
- Schlender, K. K. & Mellgren, R. L. (1984) *Proc. Soc. Exp. Biol. Med.* **177**, 17–23