REVERSIBLE INHIBITION OF ORNITHINE DECARBOXYLASE BY POLYANIONS

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1. Introduction

Ornithine decarboxylase (EC 4.1.1.17), which catalyzes the conversion of ornithine to putrescine, the rate-limiting step in the biosynthesis of the polyamines [1–5], is known to play a key role in the control of cell growth, proliferation and differentiation [6–9]. Therefore, the regulatory mechanism of its activity is of particular interest. The existence of inhibitor proteins was initially demonstrated in rat hepatoma cells [10,11] and has been amply verified in animal cells, cultured cells and *Escherichia coli* [9]. A nondialyzable activator has also been demonstrated in *E. coli* [12], suggesting the existence of a post-translational regulatory mechanism of the activity of ornithine decarboxylase. Here, the activity of ornithine decarboxylase is reported to be inhibited by polyanions including a naturally occurring polyanion such as heparin, presumably owing to a direct interaction of polyanions and the enzyme protein. The interaction of heparin and the enzyme was prevented by the addition of salts.

2. Materials and methods

D,L-[1-^{14}C]Ornithine (58 mCi/mmol) was purchased from Radiochemical Centre, Amersham. DE-52 was from Whatman Biochemicals. Sepharose 4B and dextran sulfate were obtained from Pharmacia Fine Chemicals. Poly(L-glutamic acid) (type II-B), poly(L-lysine) (type I-B), DNA (type I), RNA (type XI) and bovine serum albumin were from Sigma Chemical Co.

Ornithine decarboxylase was purified ~150-fold with a 30% yield from the liver cytosol of the rats which had been treated with thioacetamide [13] using acid treatment, ammonium sulfate fractionation, acetone fractionation and chromatography on DE-52. Enzyme activity was determined by measurement of the rate of $^{14}CO_2$ evolution from L-[1-^{14}C]ornithine. The assay mixture contained, in 0.5 ml final vol.: 50 mM sodium phosphate buffer (pH 7.0), 0.1 mM pyridoxal phosphate, 5 mM dithiothreitol, 0.6 mM L-ornithine and a suitable amount of the enzyme solution. The reaction was carried out at 37°C for 1 h with shaking. The reaction was stopped by the addition of 0.4 ml 8% perchloric acid and the $^{14}CO_2$ evolved was determined essentially as in [14].

Protein concentration was determined as in [15] with bovine serum albumin as a standard.

Heparin-substituted Sepharose 4B was prepared as in [16].

3. Results and discussion

Fig.1 shows the inhibitory effect of heparin on the activity of ornithine decarboxylase. In the presence of increasing amounts of heparin, the activity of ornithine decarboxylase was progressively inhibited. Similar inhibition of the enzyme was observed to occur by the addition of other polyanions such as poly(L-Glu) and dextran sulfate, although poly(L-Lys), a polycation, showed no significant inhibition of the enzyme as shown in table 1. DNA and RNA also appeared to elicit a slight inhibition of the enzyme.

The inhibition of the enzyme by heparin and dextran sulfate was prevented by 0.15 M NaCl, suggesting that electrostatic forces may be implicated in this inhibition. To examine whether the inhibition of the enzyme by heparin occurred as a result of a direct interaction between the enzyme and heparin or not, the interaction of ornithine decarboxylase with immobilized heparin was studied as shown in fig.2. When a partially purified preparation of ornithine decarboxyl-

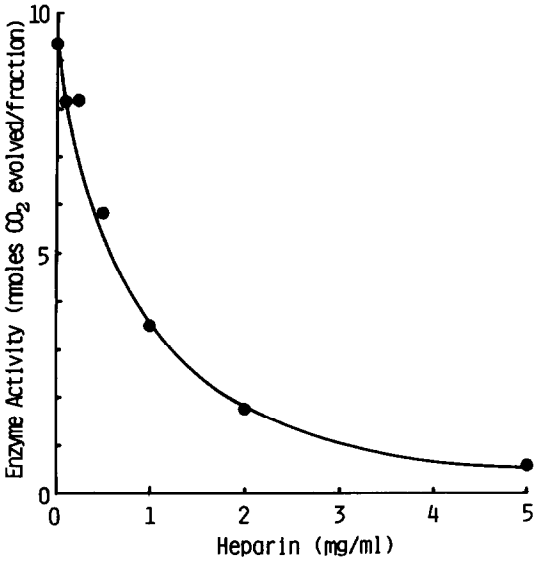

Fig.1. Effect of increasing concentrations of heparin on the inhibition of the activity of ornithine decarboxylase. The activity of ornithine decarboxylase (55.9 μg protein) was measured as in section 2 but in the presence of the indicated amounts of heparin.

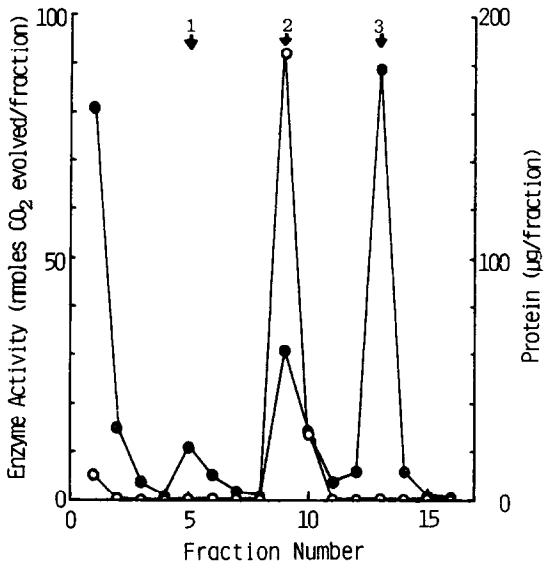

Fig.2. Elution profile of ornithine decarboxylase from heparin-substituted Sepharose. Partially purified ornithine decarboxylase (~750 μg protein) was applied to a small heparin-substituted Sepharose column (0.1 ml) equilibrated with 10 mM sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA, 10 mM 2-mercaptoethanol and 0.1 mM pyridoxal phosphate. The column was eluted successively with increasing levels of NaCl in the equilibration buffer in 0.2 ml fractions. At the points by the arrows, the NaCl level was changed: (1) 25 mM; (2) 150 mM; (3) 500 mM. Aliquots of each fraction were measured for the ornithine decarboxylase activity (○) and the protein concentration (●) as in section 2.

ase was applied to a column of heparin-substituted Sepharose, >95% of the enzyme activity was bound to the column. After washing with 25 mM NaCl in 10 mM sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA, 10 mM 2-mercaptoethanol and 0.1 mM pyridoxal phosphate, ~90% of the total activity was eluted with NaCl at the same level (0.15 M) as that at which the inhibition of the enzyme by heparin was prevented by NaCl, suggesting that the inhibition may result from a direct interaction of the enzyme with heparin and furthermore suggesting that the inhibition

Table 1
Inhibition of ornithine decarboxylase activity by polyanions

Additions	Enzyme activity (nmol $^{14}CO_2$ evolved)	(%)
None	7.30	(100)
Heparin (2 mg/ml)	1.24	(17)
Poly(L-Glu) (2 mg/ml)	0.15	(2)
Dextran sulfate (2 mg/ml)	0.60	(8)
Poly(L-Lys) (2 mg/ml)	6.93	(95)
DNA (2 mg/ml)	6.30	(86)
RNA (2 mg/ml)	4.97	(68)
NaCl (0.15 M)	5.85	(80)
Heparin + NaCl	4.23	(58)
Poly(L-Glu) + NaCl	0.20	(3)
Dextran sulfate + NaCl	3.38	(46)

may be a reversible process. Although the precise role of polyanions in modulating ornithine decarboxylase in vivo remains unknown, the fact that many tissue components, including cell membranes, are polyanionic leads to the speculation that polyanionic compounds may act as an important factor in the regulation of biosynthesis of polyamines.

The reversible binding of ornithine decarboxylase to heparin may provide a useful tool for purification of the enzyme, since the enzyme preparation in the fraction 9 which was eluted with 0.15 M NaCl from the column of heparin-substituted Sepharose (fig.2) was purified almost 10-fold with ~80% yield.

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