Properties and fluctuations *in vivo* of rat liver antizyme inhibitor

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Antizyme inhibitor was highly purified from rat liver by using affinity chromatography. It has some structural resemblance to ornithine decarboxylase (ODC), as judged from M_r , immunoreactivity and reversible binding with antizyme. However, unlike hepatic amounts of ODC and ODC–antizyme complex, that of antizyme inhibitor did not show much fluctuation upon putrescine treatment, whereas it decreased as rapidly as ODC decay in the presence of cycloheximide. These results suggested that antizyme inhibitor is an independent regulatory protein rather than a derivative of ODC. Changes in hepatic amounts of antizyme inhibitor, antizyme and ODC upon feeding suggested that antizyme inhibitor may play a role in ODC regulation by trapping antizyme and thereby suppressing ODC degradation. A monoclonal antibody to rat liver antizyme inhibitor was obtained. This antibody was shown to be utilizable for a simple assay of antizyme-inhibitor activity in tissue extracts.

INTRODUCTION

Mammalian ODC is the first and key enzyme in the synthesis of polyamines, which are essential in cellular growth and differentiation processes (Tabor & Tabor, 1984). The enzyme turns over very rapidly and is under a unique regulatory mechanism, in which a polyamine-induced protein, antizyme, binds to the enzyme and inhibits its activity (Canellakis *et al.*, 1979). More recent results have suggested that the binding with antizyme leads to a rapid degradation of ODC (Seely & Pegg, 1983; Murakami & Hayashi, 1985; Murakami *et al.*, 1988). On the other hand, we reported previously that rat liver cytosol contained another protein factor, termed ‘antizyme inhibitor’, which specifically binds to antizyme with a high affinity and thus can release active ODC from inactive ODC–antizyme complex (Fujita *et al.*, 1982).

In the present study, we studied physicochemical and immunochemical properties of highly purified antizyme inhibitor, and examined the fluctuation of tissue contents of antizyme inhibitor under various conditions in order to investigate its physiological role.

EXPERIMENTAL

Materials and animals

L-[1- 14 C]Ornithine was obtained from American Radiolabeled Chemicals, St. Louis, MO, U.S.A. α -Difluoromethylornithine was kindly given by Dr. P. P. McCann of Merrell Research Center, Cincinnati, OH, U.S.A. Hydroxyapatite was purchased from Seikagaku Kogyo Co., Tokyo, Japan. Sprague–Dawley rats and BALB/c mice were purchased from Sankyo Labo Service Co., Tokyo, Japan. Wistar rats were from Ishikawa Experimental Animals, Saitama, Japan. They were maintained on a 12 h-light/12 h-dark cycle and usually fed *ad libitum* on a stock diet (Oriental Yeast Co., Tokyo, Japan). For dietary induction of hepatic ODC, rats were meal-fed from 09:00 to 17:00 h for at least 1 week before

the experiment. Synthetic diets were prepared as previously reported (Noguchi *et al.*, 1979).

Preparations of ODC and antizyme

ODC was partially purified by DEAE-cellulose column chromatography from livers of rats injected with thioacetamide (150 mg/kg body wt., 20 h before death) and 2-aminoisobutyric acid (600 mg/kg body wt., 4 h before death) as described by Hayashi & Kameji (1983), and used in assays of antizyme and antizyme-inhibitor activities. Antizyme was partially purified by DEAE-cellulose column chromatography and Sephadex G-75 gel filtration from livers of rats injected with 1 mmol of putrescine/kg body wt. 1, 2 and 3 h before death as described by Hayashi & Fujita (1983).

Assays

ODC and antizyme activities were determined as described previously (Murakami *et al.*, 1988). One unit of ODC activity was defined as the amount forming 1 nmol of CO_2 /h. One unit of antizyme activity was defined as the amount inhibiting one unit of ODC activity. Activity of antizyme inhibitor was determined by measuring the reversal of ODC inhibition caused by antizyme, as described previously (Fujita *et al.*, 1982). One unit of antizyme inhibitor was defined as the amount reversing the inhibition of 1 unit of ODC activity by antizyme.

Purification of antizyme inhibitor from rat liver

For this, 50 Wistar rats were injected intraperitoneally with thioacetamide and 2-aminoisobutyric acid as described above. Livers were homogenized with 2 vol. of 25 mM-Tris/HCl buffer, pH 7.4, containing 0.2 M-sucrose, 1 mM-dithiothreitol and 1 mM-EDTA. The homogenate was centrifuged at 100 000 g for 60 min. The crude extract was freed from ODC by passing through a column of anti-ODC–Affi-Gel 10 (4.0 ml) prepared as described

Abbreviation used: ODC, ornithine decarboxylase.

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previously by using HO-101 monoclonal antibody to rat liver ODC (Matsufuji *et al.*, 1984), and then applied to an antizyme-loaded anti-antizyme-Affi-Gel 10 column (6.0 ml) prepared as described previously (Matsufuji *et al.*, 1984) by using HZ-3H1 monoclonal antibody to rat liver antizyme (Onoue *et al.*, 1988). After washing of the column with 25 mM-Tris/HCl, pH 7.4, containing 10 mM-mercaptoethanol and 0.01% Tween 80 (buffer A), antizyme inhibitor was eluted with 90 ml of buffer A containing 1 M-NaCl. The eluate was applied to a hydroxyapatite column (0.3 ml) that had been equilibrated with buffer A containing 1 M-NaCl. After washing of the column with buffer A, antizyme inhibitor was eluted with 0.9 ml of 0.2 M-sodium phosphate buffer, pH 7.0. The specific activity of the final preparation was not determined, since its protein content was too low to be assayed. It was observed that only 0.015% of total cytosol protein remained after the affinity chromatography, indicating that a high degree of purification had been achieved by that step. In repeated trials, about 2–4 μ g of antizyme inhibitor was usually contained in the final preparations each from 500 g of rat liver, as calculated from its activity and subunit M_r (50000), on the assumption that one molecule of antizyme-inhibitor subunit blocks the activity of one molecule of antizyme, and thus the final specific activity of antizyme inhibitor is the same as that of ODC, 1500000 units/mg of protein.

Production of mouse antiserum and monoclonal antibody against rat liver antizyme inhibitor

A BALB/c mouse was injected three times intraperitoneally each time with the affinity-purified preparation containing about 1.0 μ g of antizyme inhibitor: the first two injections were made at a 3-week interval after mixing with aluminium hydroxide gel with

and without 2×10^9 killed cells of *Bordetella pertussis*. A booster injection was made 2 weeks later without any adjuvant. The blood was collected 3 days later, and the serum was used as a polyclonal antibody to rat liver antizyme inhibitor, and the spleen was used for the production of hybridomas, from which a monoclonal antibody to rat liver antizyme inhibitor was obtained essentially by the method of Hales (1977). Thus mouse spleen cells (2.5×10^7) were fused with mouse myeloma cells (1×10^8), P3-NSI/1-Ag 4-1 (Köhler *et al.*, 1976), in the presence of poly(ethylene glycol)/dimethyl sulphoxide. One clone of hybridoma cells secreting anti-antizyme inhibitor, HI-12, was obtained after screening for the antibody titre in culture media.

Immunotitration of antizyme inhibitor and ODC with polyclonal antibodies

For examination of cross-reaction with polyclonal anti-(mouse kidney ODC) antibody (Nishiyama *et al.*, 1988), antizyme inhibitor and ODC were each incubated at room temperature for 15 min, in a total volume of 500 μ l, with 25 mg of formalin-fixed *Staphylococcus aureus* Cowan 1 cells which had bound various amounts of polyclonal anti-(mouse kidney ODC) antibody or normal IgG. After centrifugation at 12000 g for 2 min, antizyme-inhibitor or ODC activities remaining in the supernatants were assayed. For examination of cross-reaction with antiserum to rat liver antizyme inhibitor, a series of fixed amounts of antizyme inhibitor were each incubated at room temperature for 15 min, in a total volume of 50 μ l, with 4 mg of formalin-fixed *S. aureus* Cowan 1 cells which had been treated first with rabbit anti-mouse IgG and then with a mixture of mouse sera consisting of various proportions of normal serum and the antiserum to rat liver antizyme inhibitor. ODC was treated separately with normal serum or the antiserum in

Fig. 1. Immunoaffinity separation of ODC, antizyme and antizyme inhibitor in tissue extracts

Crude extract corresponding to less than 10 g of tissue was used. About 100 units of antizyme had been bound to the HZ-3H1 monoclonal antibody-Affi-Gel 10 before use. Yields are shown in parentheses.

* Free ODC plus ODC originated from ODC-antizyme complex.

† Originated from ODC-antizyme complex.

‡ Originated from antizyme-inhibitor-antizyme complex.

the same way, except that the amount of formalin-fixed *S. aureus* Cowan 1 cells used was 20 mg and total incubation volume was 100 μ l. After incubation, the pellets were removed by centrifugation at 12000 *g* for 2 min and the supernatants were assayed for remaining antizyme inhibitor or ODC activity.

Immunoprecipitation of antizyme inhibitor with monoclonal antibody

Various amounts of the HI-12 monoclonal antibody to antizyme inhibitor were each incubated in a total volume of 20 μ l with 1 mg of formalin-fixed *S. aureus* Cowan I cells which had bound 14 μ g of affinity-purified rabbit anti-(mouse IgG) antibody. The mixtures were kept at room temperature for 15 min, and then centrifuged at 12000 *g* for 2 min. The precipitates were washed with 0.5 ml of 20 mM-Tris/HCl, pH 7.5, containing 0.15 M-NaCl, and each was incubated with a fixed amount of affinity-purified antizyme inhibitor in a total volume of 50 μ l. The mixtures were kept at room temperature for 15 min and then centrifuged at 12000 *g* for 2 min. The supernatants were assayed for remaining antizyme-inhibitor activity. The precipitates were washed with 0.5 ml of 20 mM-Tris/HCl buffer, pH 7.5, containing 0.15 M-NaCl, and suspended in the assay mixture for determination of their antizyme inhibitor activity.

Immunoaffinity separation of ODC, antizyme and antizyme inhibitor in tissue extracts

Tissue amounts of antizyme and antizyme inhibitor can be determined accurately only after separation from ODC and from each other. We therefore developed an efficient method in which a series of immunoaffinity separations were used. Each tissue was homogenized with 2–4 vol. of 0.25 M-sucrose containing 1 mM-dithiothreitol. A supernatant was obtained after centrifugation at 100000 *g* for 1 h, and 10–20 ml of the supernatant was applied on an anti-ODC antibody-Affi-Gel 10 column connected with an antizyme-loaded anti-antizyme-Affi-Gel 10 column. The column sizes and chromatographic conditions are shown in Fig. 1.

RESULTS

Properties of antizyme inhibitor

Upon gel-filtration analysis the antizyme inhibitor showed a M_r of 105000 in the presence of 0.05 M-NaCl, as previously reported (Fujita *et al.*, 1982), whereas it showed a M_r of 50000 in the presence of 1 M-NaCl (cf. Fig. 2). Thus antizyme inhibitor appears to be composed of two identical subunits of M_r 50000 which are dissociable in the presence of high concentration of salt. We showed previously by gel-filtration analysis that equivalent amounts of antizyme and antizyme-inhibitor activities disappeared when the two were mixed, indicating the formation of a complex between the two (Fujita *et al.*, 1983). In the presence of high concentration of salt (1 M-NaCl), however, antizyme and antizyme inhibitor were separated on gel filtration (Fig. 2). These results indicated that antizyme-inhibitor-antizyme complex is dissociable into antizyme-inhibitor subunits and antizyme at high salt concentration.

Antizyme inhibitor cross-reacted with polyclonal anti-(mouse kidney ODC) antibody to an extent of about 2.5% as compared with ODC (Fig. 3a). Conversely, cross-reactivity of rat hepatic ODC with mouse antiserum

Fig. 2. Dissociation of antizyme-inhibitor-antizyme complex in the presence of 1 M-NaCl

Antizyme (23 units) and antizyme inhibitor (29 units) preparations were mixed in a total volume of 0.5 ml and applied to a column of TSKgel G3000SW (7.5 mm $\times$ 60 mm; Toso, Tokyo, Japan) in the presence of 1 M-NaCl. The mixture had no free antizyme activity before application to the column. The column was eluted with buffer A containing 1 M-NaCl in 0.86 ml fractions. A portion of each fraction was assayed for both antizyme (AZ) activity and antizyme inhibitor (AI) activity by adding to a mixture of 9.6 units of ODC and 4.4 units of antizyme and measuring a decrease or an increase, respectively, in ODC activity compared with the control value, indicated by a horizontal line (5.2 units). Standard proteins were eluted under the same conditions: 1, bovine serum albumin (M_r 67000); 2, ovalbumin (M_r 43000); 3, cytochrome *c* (M_r 12400).

raised against rat hepatic antizyme inhibitor was 1% as compared with antizyme inhibitor (Fig. 3b). These results suggested some structural resemblance between antizyme inhibitor and ODC.

Presence of antizyme inhibitor in various tissues

Tissue contents of ODC, ODC-antizyme complex and antizyme inhibitor were determined after their separation from each other by a series of immunoaffinity chromatographies (Fig. 1). In this method we used a monoclonal anti-antizyme-Affi-Gel 10 column which had been partially saturated with antizyme and therefore could trap both free antizyme inhibitor and antizyme-inhibitor-antizyme complex. The antizyme loading was not necessarily required, however, since about the same amounts of antizyme inhibitor were recovered when crude tissue extracts were applied to the affinity columns with and without loaded antizyme. This indicated that antizyme inhibitor in crude extracts is mostly present as

Fig. 3. Cross-reactions between antizyme inhibitor and anti-ODC polyclonal antibody (a) and between ODC and anti-antizyme-inhibitor antiserum (b)

(a) ODC and antizyme inhibitor were each titrated with anti-ODC polyclonal antibody (Δ , $\circ$) or control IgG ($\blacktriangle$, $\bullet$). (b) ODC was titrated with antiserum against rat liver antizyme inhibitor (Δ) or normal serum ($\blacktriangle$). Antizyme inhibitor was titrated with the antiserum under the condition in which total amount of serum was adjusted to be constant by using normal mouse serum in order to subtract any non-specific inhibition by serum. The detailed procedure is described in the Experimental section.

antizyme-inhibitor-antizyme complex. As shown in Table 1, antizyme inhibitor was present in various amounts in all the tissues examined. Fairly large amounts of antizyme inhibitor were present in the liver and heart of the rat in which ODC activity had been induced, whereas only small amounts were present in male mouse and rat kidneys, which contained high ODC activity. Thus tissue contents of antizyme inhibitor were not

proportional to that of ODC and, as also shown in Table 1, it was not proportional to the amount of ODC-antizyme complex, either.

Changes in hepatic content of antizyme inhibitor under various conditions

Dietary protein intake induces hepatic ODC activity in rats (Noguchi *et al.*, 1979). We compared changes in hepatic contents of antizyme inhibitor with those of ODC-antizyme complex and ODC activity after feeding. As shown in Fig. 4, antizyme inhibitor increased 2.5-fold to reach a maximum at around 4 h after feeding, coinciding with the peak of ODC activity. The degree of increase, however, was much less for antizyme inhibitor than for ODC activity. On the other hand, ODC-antizyme complex decreased transiently, and the amount of total antizyme, which was the sum of ODC-antizyme and antizyme inhibitor (complexed with antizyme), remained almost the same for 4 h. These results therefore suggest that antizyme inhibitor may participate in dietary induction of ODC by trapping antizyme and thereby stabilizing ODC.

Dietary induction of ODC in rat liver depended on the quality of protein in the diet (Noguchi *et al.*, 1979). Zein did not induce ODC unless it was supplemented with the deficient amino acids, tryptophan and lysine. The response of hepatic antizyme inhibitor to diet was different from that of ODC activity, as shown in Table 2. Antizyme inhibitor was increased by dietary protein, regardless of its nutritive value. This suggests that the signal for induction is different for ODC and for antizyme inhibitor, although other explanations are also possible.

We next examined effects of putrescine and/or cycloheximide on hepatic contents of ODC, ODC-antizyme complex and antizyme inhibitor. As shown in Fig. 5, antizyme inhibitor decayed as rapidly as ODC after treatment with cycloheximide. On the other hand, antizyme-inhibitor amount remained almost the same for 1 h after treatment with putrescine, whereas ODC-antizyme complex increased 6-fold and free ODC almost disappeared during the same period. These results suggest that antizyme inhibitor is formed by synthesis *de novo* rather than by modification of preformed ODC of either free or complexed type. The effects of putrescine on ODC and ODC-antizyme complex were not manifest when cycloheximide was added simultaneously, as reported previously (Seely & Pegg, 1983; Mitchell *et al.*, 1985; Murakami *et al.*, 1985).

Preparation of a monoclonal antibody to rat liver antizyme inhibitor

A clone of hybridoma cells secreting an antibody to antizyme inhibitor was obtained as described in the Experimental section. The antibody was identified as IgG₃ of κ -type by Ouchterlony double-immunodiffusion analysis (result not shown). The monoclonal antibody could precipitate liver antizyme inhibitor in a dose-dependent manner up to more than 95% in the presence of formalin-fixed *S. aureus* Cowan 1 cells which had bound second antibody (rabbit anti-mouse IgG), as shown in Fig. 6, and on average about 60% of antizyme-inhibitor activity that disappeared from supernatants was detectable in precipitates. On the other hand, the monoclonal antibody did not cross-react with rat liver ODC. These results suggested that this monoclonal antibody is useful to concentrate and determine antizyme

Table 1. Antizyme-inhibitor activity in various tissues

Animals were treated with the following compounds at the indicated doses per kg body wt. and for the indicated durations: thioacetamide (150 mg, 22 h), 2-aminoisobutyric acid (AIB, 600 mg, 4 h), α -difluoromethylornithine (DFMO, 2% in drinking water, 5 days), isoprenaline (10 mg, 5 h). Values were obtained for pooled tissue extracts. Numbers of animals used (*n*) are indicated in parentheses. In some cases in which experiments were done in three or four subgroups of animals, results were expressed as means $\pm$ S.D. ND, not determined.

Tissues	(<i>n</i>)	Free ODC (units/g)	ODC-antizyme complex (units/g)	Antizyme inhibitor (units/g)
Rat liver				
Starved, overnight	(14)	3.3 $\pm$ 2.4	6.0 $\pm$ 1.3	2.2 $\pm$ 0.6
Fed, 4 h	(9)	96 $\pm$ 10	2.5 $\pm$ 1.7	5.9 $\pm$ 1.3
Thioacetamide	(11)	195 $\pm$ 94	6.4 $\pm$ 3.4	7.9 $\pm$ 1.2
Thioacetamide + AIB	(151)*	448 $\pm$ 230	8.6 $\pm$ 5.0	11.0 $\pm$ 3.4
DFMO	(15)	1.4†	3.4	0.9
Rat kidney, fed 4 h	(4)	295	36.9	0.6
Rat heart, isoprenaline	(49)	175	2.2	11.8
Mouse liver	(13)	0	1.6	1.6
Mouse kidney (male)	(13)	2570	78.2	1.3
Mouse brain	(13)	0	3.9	0.5
Calf liver	(1)	0.3	ND	2.2

* Originally used for the purification of antizyme inhibitor.

† Inactivated ODC was present at a level of about 20 unit equivalents/g tissue.

inhibitor in crude tissue extracts. Various amounts of crude extracts were each incubated in the presence of 1 M-NaCl with 10 μ l of suspension of the monoclonal antibody (1 μ g)-anti-mouse IgG (14 μ g)-Cowan 1 cells

Fig. 4. Changes in hepatic contents of ODC, ODC-antizyme complex and antizyme inhibitor after feeding

Meal-fed rats were killed at the indicated times after feeding with stock diets. Results were means $\pm$ S.E.M. for three to nine separate experiments, except for the value for ODC-antizyme complex 6 h after feeding, which was the mean of two experiments. In each experiment liver extracts from one to four rats were pooled. Contents of antizyme and antizyme inhibitor were determined after separation from ODC and from each other by a series of immunoaffinity chromatographies as shown in Fig. 1. A similar time course was obtained in another experiment with rats fed on 50%-casein diet. ●, ODC activity; ■, ODC-antizyme complex; ○, antizyme inhibitor.

(1 mg) prepared as described in the Experimental section. Antizyme-inhibitor activity recovered in the precipitates was proportional to the amount of crude extract used, ranging up to 0.5 ml. By this method we obtained results similar to that shown in Table 2 and Fig. 5. This method could also be applied at least to rat heart and kidney antizyme inhibitors (results not shown).

DISCUSSION

We previously reported the presence in rat liver of a macromolecular inhibitor of antizyme which binds strongly to antizyme and liberates free ODC from ODC-antizyme complex by replacement. This protein, named 'antizyme inhibitor', resembled ODC in its molecular size. In the present paper, we confirmed that antizyme inhibitor has some additional structural resemblance to ODC, such as immunoreactivity and reversible binding with antizyme. These results tempted

Table 2. Different responses of hepatic ODC and antizyme inhibitor to various diets

Rats that had been trained to meal-feeding were starved for 1 day and divided into four groups. One group was killed before feeding, and other groups were given test diets and killed 4 h later. Results are means $\pm$ S.E.M. for four or eight rats.

Diet	<i>n</i>	Activity (units/g of liver)	
		ODC	Antizyme inhibitor
Starved	8	4.7 $\pm$ 0.8	1.1 $\pm$ 0.1
Non-protein	4	6.0 $\pm$ 1.4	1.0 $\pm$ 0.2
Zein	4	7.3 $\pm$ 1.9	2.2 $\pm$ 0.2
Casein	8	53.2 $\pm$ 5.5	2.3 $\pm$ 0.2

Fig. 5. Effects of putrescine and/or cycloheximide on hepatic contents of ODC, ODC-antizyme complex and antizyme inhibitor

Rats were first injected intraperitoneally with thioacetamide for induction of hepatic ODC. After 22 h they were injected with putrescine (Put, 1 mmol/kg body wt.) and/or cycloheximide (CH, 50 mg/kg body wt.) and killed at the indicated times. Liver extracts from each group of four rats were pooled. Contents of antizyme and antizyme inhibitor were determined after separation from ODC and from each other by a series of immunoaffinity chromatographies as shown in Fig. 1.

Fig. 6. Immunoprecipitation of rat liver antizyme inhibitor with monoclonal antibody to rat liver antizyme inhibitor

A series of fixed amounts of antizyme inhibitor were incubated with various amounts of the monoclonal antibody to antizyme inhibitor which had been bound to anti-(mouse IgG)-Cowan I cells. After centrifugation the supernatants were assayed for remaining antizyme-inhibitor activity, and the washed precipitates were suspended in assay mixture and determined for antizyme inhibitor activity. ○, Supernatant; ●, precipitates.

a speculation that antizyme inhibitor is a post-translationally modified product of ODC or an intermediate formed in the process of ODC degradation. In these cases, cellular content of antizyme inhibitor is expected to vary in parallel with ODC or ODC-antizyme complex, respectively. However, hepatic antizyme-inhibitor content was found to fluctuate independently of ODC or ODC-antizyme complex upon putrescine treatment (Fig. 5) and upon intake of zein diet (Table 2). Furthermore, antizyme inhibitor immediately started to decay at a rapid rate upon cycloheximide treatment. These results suggest that antizyme inhibitor is an independent regulatory protein rather than a derivative of ODC.

ODC is known to be induced in response to a wide variety of stimuli within a short period of time (Tabor & Tabor, 1984). It has been found that both an increase in synthesis rate and a decrease in degradation rate are involved in ODC induction (Kanamoto *et al.*, 1986; Hölttä & Pohjanpelto, 1986). The mechanism by which degradation of ODC is regulated is not well understood. We have observed a good correlation between ODC decay rate in the presence of cycloheximide and the proportion of ODC present as a complex with antizyme (Murakami & Hayashi, 1985). It was noticed that the proportion of cellular ODC that is complexed with antizyme becomes smaller during the early phase of ODC induction, since the increase in ODC is faster than that in antizyme which is induced by the product of ODC (Murakami *et al.*, 1985). The present results showed that not only the proportion but also the amount of ODC-antizyme complex decreased, mostly owing to an increase in the amount of antizyme inhibitor during the early phase of ODC induction (Fig. 4). It is likely that antizyme inhibitor stabilizes ODC by trapping antizyme

during the phase of ODC increase after growth stimuli, whereas antizyme accelerates ODC degradation during the phase of ODC decline. It should be noted in this connection that the increase in the amount of antizyme inhibitor was comparable with the amount of ODC-antizyme complex present at that time. A temporary decrease in the amount of ODC-antizyme complex after stimulation of ODC was also reported in rat heart by Flamigni *et al.* (1986), who proposed a participation of antizyme inhibitor as a possible reason for the decrease. In fact, we confirmed the presence in rat heart of a relatively large amount of antizyme inhibitor, based on its kinetic properties, and a transient increase in its amount after stimulation of ODC induction by isoprenaline (results not shown).

Antizyme inhibitor was detectable in various animal tissues, as shown in Table 1, but the contents are too small to be radio-labelled *in vivo* for study of its metabolism. Cloning of cDNA for rat liver antizyme inhibitor is needed to clarify its primary structure as well as its role in ODC regulation. Our monoclonal antibody should be useful not only for the determination of antizyme inhibitor in crude extract as described above but also for the cloning of its cDNA.

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