Ornithine decarboxylase antizyme in kidneys of male and female mice

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Antizyme, a protein inhibitor of ornithine decarboxylase (ODC), was shown to be induced in mouse kidney by repeated injection of putrescine. Antizyme was also present as a complex with ODC in the kidney of untreated mouse. The amount of the renal ODC–antizyme complex was 3-fold higher in male mice than in female mice. On the contrary, the proportion of ODC present as a complex with antizyme was 24-fold higher in females than in males, and the decay of renal ODC activity after cycloheximide treatment was about 5-fold more rapid in females than in males. Administration of testosterone to female mice, a procedure known to prolong the half-life of renal ODC, increased both ODC activity and the content of ODC–antizyme complex, but decreased the antizyme/ODC ratio in the kidney. These results are consistent with the previous observation in HTC cells that the decay rate of ODC activity in the presence of cycloheximide correlated well with the proportion of ODC present as a complex with antizyme, suggesting the ubiquitous role of antizyme in ODC degradation.

INTRODUCTION

Ornithine decarboxylase (ODC), a key enzyme in biosynthesis of polyamines, has an extremely short half-life in eukaryotic cells, and is regulated by putrescine, spermidine or spermine, the direct or indirect product of the enzyme (reviewed by Pegg, 1986). Thus exogenously added polyamines abolish the induction of ODC and cause a rapid decrease of the ODC activity that is present. The polyamine effect appears to depend on at least three mechanisms, including the inhibition of ODC synthesis, acceleration of enzyme degradation and induction of an inhibitory protein named ‘antizyme’, which binds to ODC stoichiometrically to form an inactive ODC–antizyme complex (Heller *et al.*, 1976; Canellakis *et al.*, 1979). Since the acceleration of ODC degradation by polyamines is blocked by cycloheximide (Seely & Pegg, 1983a; Mitchell *et al.*, 1985; Murakami *et al.*, 1985), the possible involvement of antizyme in that process has been suggested. We have found the presence of ODC–antizyme complex in HTC cells under physiological conditions without exogenous polyamines (Murakami *et al.*, 1985) and the existence of a good correlation between the reciprocal of the half-life of ODC in the presence of cycloheximide and the proportion of ODC present as a complex with antizyme (Murakami & Hayashi, 1985). We also found an extraordinary accumulation of ODC–antizyme complex in ODC-stabilized variant HMO_A cells (Murakami *et al.*, 1985). These observations suggest that antizyme plays a key role in degradation of ODC *in vivo*. If this is the case, antizyme should be present in all tissues or cells containing rapidly turning-over ODC. Mouse kidney is one of the tissues that have been used most extensively for studies on ODC, owing to the high ODC activity in males and marked inducibility of ODC by excess androgens. Antizyme, however, has not been found in mouse kidney,

even after treatment with putrescine or its analogue (Pegg *et al.*, 1978; Persson & Rosengren, 1979; Seely & Pegg, 1983b; Laitinen *et al.*, 1985), despite the short half-life of renal ODC and its rapid decay upon diamine treatment (Persson *et al.*, 1984). The difficulty of finding antizyme in the kidney may have been due, in part, to rapid excretion of polyamines from the tissue (Pegg *et al.*, 1978; Persson, 1981a; Seely & Pegg, 1983b). In fact, we previously found that antizyme is present in RK 13 rabbit kidney cells in culture, where extracellular putrescine can be maintained at high concentrations, and that ODC–antizyme complex is present even in untreated RK 13 cells (Murakami *et al.*, 1986). In the present study, we have demonstrated that mouse kidney antizyme is not only inducible by exogenous putrescine but also present as ODC–antizyme complex under physiological conditions. We further compared polyamine contents, antizyme/ODC ratio and ODC decay rate between male and female mouse kidneys in order to assess the physiological role of renal antizyme.

EXPERIMENTAL

Materials

DL-[1-¹⁴C]Ornithine (59 Ci/mol) was obtained from New England Nuclear Corp.; DEAE-Cellulofine was from Seikagaku-Kogyo Co., Tokyo, Japan; testosterone enanthate was from Teikoku-Zoki Co., Tokyo, Japan; Affi-Gel 10 was from Bio-Rad Laboratories, Richmond, CA, U.S.A.; and all other chemicals were obtained from Nakarai Chemicals, Kyoto, Japan.

Animals

ICR mice and Sprague–Dawley rats were purchased from Sankyo Labo Service Co., Tokyo, Japan. Animals were maintained on a 12 h-light/12 h-dark cycle and fed *ad libitum* on laboratory chow.

Abbreviation used: ODC, ornithine decarboxylase (EC 4.1.1.17).

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Preparation of ODC and antizyme inhibitor

ODC and antizyme inhibitor were prepared from livers of rats injected intraperitoneally with thioacetamide (150 mg/kg body wt., 20 h before being killed) and α -aminoisobutyric acid (600 mg/kg, 4 h before being killed). ODC was partially purified by DEAE-cellulose column chromatography as described previously (Kameji *et al.*, 1982). Antizyme inhibitor was prepared as follows. Liver extract (550 g of liver) was applied to a DEAE-cellulose column (800 ml; 4 cm $\times$ 64 cm) and active fractions were pooled and fractionated with $(\text{NH}_4)_2\text{SO}_4$ (0–65 % saturation) as described previously (Hayashi & Fujita, 1983). Dialysed sample was filtered through a column of anti-ODC-Affi-Gel 10 (1 ml) prepared as described previously (Matsufuji *et al.*, 1984) to remove ODC from the sample, and effluent was applied to a DEAE-Cellulofine column (10 ml; 1.6 cm $\times$ 5.2 cm) equilibrated with 25 mM-Tris/HCl, pH 7.2, containing 1 mM-dithiothreitol and 0.01 % Tween 80. After washing with the same buffer, antizyme inhibitor was eluted from the column with 0.4 M-NaCl in the same buffer and collected in 10 ml fractions; 1800 units of antizyme inhibitor/20 ml was obtained. One unit of antizyme inhibitor is defined as the amount reversing the inhibition of one unit of ODC activity by antizyme. The inhibitor was stable even after freeze-thawing several times.

Assays

ODC activity was assayed as described previously (Murakami *et al.*, 1984), except that the concentration of pyridoxal phosphate in the reaction mixture was 40 μM and Tween 80 was added at a final concentration of 0.01 %. Total volume of the assay mixture was 1.25 ml. In some studies, ODC was assayed by a more sensitive method in which the specific radioactivity of the substrate was increased 10-fold (micro-assay system). In this assay method, the reaction was carried out in a final volume of 125 μl (one-tenth the volume used in the previous assay method) in a 1.5 ml Eppendorf 3810 micro-test tube with a filter paper attached to the inside of the cap. The filter paper was moistened with 10 μl of 10 % (w/v) KOH. After incubation, the cap was opened for a moment to add 50 μl of 6 M-HCl. Radioactivity of the filter paper was determined after more than 15 min incubation at 37 °C. We confirmed that the two methods gave identical ODC activity. One unit of enzyme activity is defined as the amount releasing 1 nmol of CO_2/h at 37 °C under the specified conditions. Antizyme (or renal inhibitor) activity was determined by measuring inhibitory activity to ODC. The standard assay was carried out in the assay mixture for ODC in the presence of 8–20 units of ODC (0.8–2.0 units in the micro assay). One unit of the activity is defined as the amount inhibiting one unit of ODC activity. The amount of ODC-antizyme complex was determined by two methods. In the competitive assay method described previously (Murakami *et al.*, 1985), α -difluoromethylornithine-inactivated ODC was used to release active ODC competitively from the complex. In the other method, the amount of the complex was determined as the increase in ODC activity caused by an addition of an excess amount of antizyme inhibitor (Hayashi & Fujita, 1983). One unit of the amount of ODC-antizyme complex is defined as the amount consisting of one unit each of ODC activity and antizyme activity. In addition to these methods, the amount of

ODC-antizyme complex was also determined in some studies as antizyme activity after dissociation and separation of the complex into the components, as follows. Crude extracts containing ODC-antizyme complex were filtered through a column of anti-ODC-Affi-Gel 10 4B to trap ODC-antizyme complex. The column was washed with 25 mM-Tris/HCl, pH 7.2, containing 10 mM-mercaptoethanol and 0.01 % Tween 80, and antizyme was then eluted, by dissociation from ODC, with 4 M-NaCl in the above buffer. When high ODC activity was contained in the crude extract, a small part of ODC was sometimes eluted as ODC-antizyme complex under the above conditions. Therefore antizyme activity in the eluates was indirectly determined by antizyme inhibitor. Thus the eluate was added to the ODC assay mixture containing a fixed amount of ODC with or without excess antizyme inhibitor, and the antizyme activity was determined from the increase in ODC activity caused by antizyme inhibitor. The yield of antizyme from a column was usually 80 %. A part of each kidney homogenate was extracted with 2 % (w/v) HClO_4 and analysed for polyamines by h.p.l.c. (Shimazu LC-5A).

Fig. 1. Inhibitory activity of kidney extracts from putrescine-treated female mice

Mice were given three consecutive injections each of 2 mmol of putrescine/kg body wt. 1, 2 and 3 h before death. Kidney extracts were prepared with 0.25 M-sucrose (●) or 0.155 M-KCl (○) as described in the Experimental section, and assayed for ODC-inhibitory activity in the micro-assay system for ODC. ODC-inhibitory activity was 66 units/g of kidney for the sucrose extract and 153 units/g of kidney for the KCl extract.

Partial purification of renal ODC inhibitor

Female or male mice were given intraperitoneal injections of putrescine 1, 2 and 3 h before being killed (2 mmol/kg each time). Kidneys were homogenized with 4 vol. of 0.25 M-sucrose or 0.155 M-KCl each containing 1 mM-dithiothreitol in a Dounce-type all-glass homogenizer. The homogenate was centrifuged at 105000 *g* for 60 min, and the supernatant was applied to a DEAE-Cellulofine column (0.25 ml/10 mg of protein) equilibrated with 25 mM-Tris/HCl, pH 7.9, containing 10 mM-mercaptoethanol. The column was washed with the same buffer. The inhibitor was eluted with 25 mM-Tris/HCl, pH 7.2, containing 10 mM-mercaptoethanol and 0.4 M-NaCl (3.5 vol. of column size) and used as a partially purified renal inhibitor preparation.

RESULTS

Identification of antizyme present in kidney extracts of putrescine-treated mice

Female mice were starved overnight to suppress renal ODC activity, and then given putrescine intraperitoneally three times. A kidney from each mouse was extracted with iso-osmotic sucrose (0.25 M), and another with iso-osmotic KCl (0.155 M). As shown in Fig. 1, the kidney extracts inhibited ODC activity with linear dose/effect relationships. Such a stoichiometric inhibitory action is characteristic of antizyme. Moreover, the inhibition was time-independent (result not shown), indicating that the inhibitor, like antizyme (Fong *et al.*, 1976; Heller *et al.*, 1976), is not an enzyme attacking ODC. The inhibitory

activity of the KCl extract was about twice as high as that of the sucrose extract, as previously observed in RK 13 rabbit kidney cells (Murakami *et al.*, 1986). The inhibitory activity of the KCl extract of kidney was roughly half of that of putrescine-treated rat liver (307 ± 71 units/g of liver).

The inhibitor was partially purified from the renal extract by DEAE-Cellulofine chromatography as described in the Experimental section, and analysed by gel filtration. As shown in Fig. 2(a), the inhibitory activity was eluted from the column at a position approximately corresponding to a M_r of 27000, which was close to the reported M_r of 26500 for antizyme from a variety of cells (Heller *et al.*, 1976). We also confirmed that the renal inhibitor was labile to heat treatment at 55 °C for 30 min, and the inhibition was completely reversed by addition of antizyme inhibitor (result not shown), a protein factor which can bind to antizyme with high affinity and thus replace ODC in the ODC-antizyme complex (Fujita *et al.*, 1982). Taken together, these results firmly identified the inhibitor as ODC antizyme.

Presence of ODC-antizyme complex in mouse kidney under physiological conditions

We examined whether antizyme is present in mouse kidney under physiological conditions, namely without exogenous polyamines. Extracts were prepared from kidneys of untreated female mice with iso-osmotic sucrose or KCl, and assayed for ODC activity in the presence and absence of antizyme inhibitor. As shown in Table 1, ODC activity was higher in the presence of antizyme inhibitor than in its absence, suggesting the

Fig. 2. Chromatography of NaCl-treated extract from female mouse kidney

Kidney extracts were prepared with 0.25 M-sucrose containing 1 mM-dithiothreitol from putrescine-treated (a) and untreated (b) female mice and partially purified by DEAE-Cellulofine chromatography as described in the Experimental section. Samples containing either 27.4 units of ODC inhibitor (a) or 6.2 units of ODC activity (b) were chromatographed on a TSKgel G3000SW column (7.5 mm $\times$ 600 mm) in the presence of 1 M-NaCl; 0.37 ml fractions were collected, and a 0.2 ml portion of each fraction was assayed for ODC activity in the presence of 10.8 units (a) or 15.1 units (b) of partially purified ODC (indicated by broken line). Collectively, 16.9 units of inhibitory activity were recovered in (a), and 28.6 units of ODC and 16.1 units of antizyme were recovered in (b). Standard proteins (indicated by arrows) were eluted under the same conditions: BSA, bovine serum albumin (M_r 67500); OVA, ovalbumin (M_r 43000); Cyt c, cytochrome c (M_r 12400).

Table 1. Effect of antizyme inhibitor on ODC activity in kidney extracts of untreated female mice

Control female mice were killed by decapitation, and a kidney of each mouse was extracted with 4 vol. of 0.25 M-sucrose and another kidney with 0.155 M-KCl, each containing 1 mM-dithiothreitol. A 55 μ l portion of each extract was assayed for ODC activity in the presence and absence of excess antizyme inhibitor (1.0 unit) in the micro-assay system. Results are means $\pm$ S.D. for four mice.

Extract	ODC activity (units/g kidney)		
	No antizyme inhibitor (A)	+ Antizyme inhibitor (B)	(B) - (A)
KCl	105 $\pm$ 25	145 $\pm$ 25	40 $\pm$ 6
Sucrose	93 $\pm$ 21	123 $\pm$ 22	31 $\pm$ 5

Table 2. Effect of testosterone on ODC activity and the amount of ODC-antizyme complex in female mouse kidney

Female mice were given a single subcutaneous injection of testosterone enanthate (25 mg/mouse) and killed at the indicated time intervals. Putrescine was injected intraperitoneally at a dose of 2 mmol/kg 30 or 60 min before death. Crude kidney extracts were prepared with 0.25 M-sucrose containing 1 mM-dithiothreitol. A part of each extract was used for assay of ODC activity. The remaining part was passed through pyridoxamine phosphate-succinyl-di(aminopropyl)amino-Sepharose 4B to remove free ODC, and assayed for the amount of ODC-antizyme complex by the competitive method with difluoromethyl-ornithine-inactivated ODC. The results were averages of two to four mice or of two experiments using pooled extracts of four mice.

Treatment	Duration	Activity (units/g of kidney)	
		ODC	ODC-antizyme complex
None	—	96	30
Testosterone	1 day	1760	19
	2 days	2018	86
	9 days	21 132	93
	11 days	15479	93
Testosterone plus putrescine	2 days + 30 min	947	162
	9 days + 60 min	15012	165

presence of ODC-antizyme complex in the kidney extracts. The amount of the complex was about 25 % of total ODC in these kidney extracts. In contrast with renal free antizyme, ODC-antizyme complex could be extracted from the kidney to about the same extent with either sucrose or KCl. The complex was partially purified by DEAE-Cellulofine chromatography essentially as described in the Experimental section for renal ODC inhibitor, and analysed by gel filtration in the presence of 1 M-NaCl, where ODC-antizyme complex can be dissoci-

ated and the components can be resolved. As shown in Fig. 2(b), both ODC and antizyme activities were eluted from the column at positions corresponding approximately to M_r 50000 and M_r 27000 respectively. This confirmed the presence of ODC-antizyme complex in the mouse kidney. Similarly, we also confirmed the presence of the complex in rat kidney (37 units/g).

Mouse kidney ODC is known to be dependent on androgens, increasing in response to treatment with testosterone (Seely *et al.*, 1982; Isomaa *et al.*, 1983). We examined the effect of testosterone on the renal content of ODC-antizyme complex in female mice. As shown in Table 2, testosterone caused progressive increases in the amount of ODC-antizyme complex. However, the increase in ODC-antizyme complex was much smaller than that in ODC activity, and appeared to reach a maximum earlier than ODC activity did. Therefore the proportion of ODC present as a complex with antizyme decreased progressively after the treatment. This should result in prolongation of half-life of ODC activity, if antizyme/ODC ratio correlated with the half-life of ODC in mouse kidney, as demonstrated in HTC cells (Murakami *et al.*, 1985). In fact, Loeb *et al.* (1984) have shown progressive prolongation of half-life of ODC activity in female mice with time after androgen treatment. Similar prolongation of the half-life of ODC activity was also observed by other groups in the kidney of female or male mice several days after administration of androgen (Seely *et al.*, 1982; Isomaa *et al.*, 1983). Administration of putrescine to testosterone-treated mice further increased the amount of ODC-antizyme complex concomitantly with some decrease in ODC activity (Table 2).

Relationship between cellular polyamine contents, free and complexed ODC activities and turnover rate of ODC in the kidneys of male and female mice

Renal ODC activity of the mouse is known to be much higher in males than in females (Henningsson & Rosengren, 1975). We examined sex differences of ODC activity, cellular polyamine contents, amount of ODC-antizyme complex and decay rates of ODC and ODC-antizyme complex in the kidneys of untreated mice. As shown in Table 3, renal ODC activity of males was about 100 times as high as that of females, whereas ODC-antizyme complex was only 3-fold higher in males. Therefore the antizyme/ODC ratio was about 25 times higher in females (0.40) than in males (0.017). On the other hand, the half-life of renal ODC activity was about 5-fold longer in males than in females. These results were consistent with the previous results obtained with HTC cells, that the decay rate of ODC activity in the presence of cycloheximide correlated with the proportion of ODC present as a complex with antizyme. In HTC cells an increase in antizyme/ODC ratio from 0.017 to 0.40 would bring about a 4-fold increase in the decay rate of ODC activity (Murakami *et al.* 1985). The above results therefore strongly suggest that antizyme plays a key role in ODC degradation in mouse kidney, too. The decay rate of ODC-antizyme complex was also about 4-fold higher in females than in males. The 3-fold larger amount of ODC-antizyme complex in males, compared with females, could therefore be attributed solely to the stabilization of antizyme, and the synthesis rate of renal antizyme is presumably the same in males and females. In spite of the large sex difference in ODC activity, the

Table 3. ODC activities, amounts of ODC-antizyme complex and their half-lives in male and female mouse kidneys

Extracts were prepared with iso-osmotic sucrose from kidneys of male and female mice before and 20 min after an intraperitoneal injection of cycloheximide (50 mg/kg body wt.) and assayed for ODC activity in the micro-assay system. Results are means $\pm$ S.D. for five mice. Equal amounts of crude extracts were pooled from five mice of each group, and the amount of ODC-antizyme complex was determined as antizyme activity after dissociation and separation of the complex into the components as described in the Experimental section. Essentially similar results were obtained in two more experiments.

	Male	Female
ODC activity (units/g of kidney)		
Before cycloheximide	4639 $\pm$ 2119	37.0 $\pm$ 11.8
20 min after cycloheximide	3214 $\pm$ 1658	6.7 $\pm$ 2.7
$t_{1/2}$ for ODC activity (min)	38	8
ODC-antizyme complex (units/g of kidney)		
Before cycloheximide	73.6	24.2
20 min after cycloheximide	61.0	11.7
$t_{1/2}$ for ODC-antizyme complex (min)	74	19

Table 4. Renal polyamine contents in male and female mice

Results are means $\pm$ S.D. for nine mice in two separate experiments.

Mouse	Polyamine (nmol/g of kidney)		
	Putrescine	Spermidine	Spermine
Male	123 $\pm$ 24	367 $\pm$ 29	775 $\pm$ 61
Female	61 $\pm$ 11	371 $\pm$ 43	790 $\pm$ 74

sex difference in renal polyamine contents was surprisingly small (Table 4). Henningson & Rosengren (1975) have shown no sex difference in renal contents of putrescine, spermidine and spermine. Our results showed that renal putrescine content in male mice was only about twice as high as that in female mice, and both spermidine and spermine contents were about the same in males and females. These results, together with those shown in Table 3, suggested that the 2-fold difference in putrescine content was not sufficient to affect antizyme synthesis in mouse kidney.

DISCUSSION

The present results clearly demonstrated that antizyme activity is inducible in the mouse kidney after administration of putrescine, and that antizyme is also present as a complex with ODC even under physiological conditions. As previously observed in cultured rabbit kidney cells, a substantial portion of antizyme in mouse kidney was bound to the particulate fraction under low-ionic-strength conditions (0.25 M-sucrose), and this could be

extracted with iso-osmotic KCl solution (Fig. 1). Besides, a fairly large amount of inhibitory activity was extracted with 1 M-NaCl from the particulate fraction of mouse kidney, even after the KCl extraction. This tightly bound inhibitor was also identified as antizyme by the time-independence of the inhibition, reversibility by antizyme inhibitor and its M_r value (results not shown). In contrast with the existence of only a small amount of bound antizyme in hepatoma cells (Heller *et al.*, 1977; McCann *et al.*, 1979) and rat liver (Heller *et al.*, 1977), the large antizyme-binding capacity of subcellular particles seems to be characteristic of the kidney and may be one of the reasons for the previous difficulties in detecting antizyme activity in mouse kidney. Rapid excretion of polyamines from kidney cells may be another reason for that (Pegg *et al.*, 1978; Persson, 1981a; Seely & Pegg, 1983b). In addition, high ODC activity in male mouse kidney may prevent overshoot of free antizyme by neutralization. Even under these conditions, however, ODC-antizyme complex may be easily detected. In fact, the present results indicate that the complex in kidney of the testosterone-treated mouse increased after administration of putrescine (Table 2). In this connection, the amount of the complex in untreated mouse kidney (Table 1) was about 4 times as large as that in untreated rat liver (7.7 ± 0.6 units/g for seven rats). Occurrence of ODC-antizyme complex as a normal cell component has been demonstrated in several tissues, including mouse brain (Hietala, 1983; Onoue *et al.*, 1988), rat liver (Fujita *et al.*, 1984), rat heart (Flamigni *et al.*, 1986) and cultured cells (McCann *et al.*, 1979; Murakami *et al.*, 1985, 1986). In addition, we confirmed it in mouse kidney as well as in rat kidney by the present study. This indicates that antizyme is present in rodent tissues ubiquitously, presumably being synthesized in response to endogenously formed polyamines.

Next, we examined sexual differences in ODC decay rate and antizyme/ODC ratio in order to assess the physiological role of renal antizyme. The mouse kidney ODC is known to be androgen-dependent, being low in females and high in males (Grahn *et al.*, 1973; Henningson & Rosengren, 1975; Persson, 1981b; Seely *et al.*, 1982; Isomaa *et al.*, 1983). The sex difference in ODC activity may depend on differences in both synthesis rate and degradation rate of the enzyme. Loeb *et al.* (1984) reported that the half-life of ODC activity *in vivo* was slightly longer in males than in females. Our results clearly showed that ODC decays more rapidly in females than in males on cycloheximide treatment. The antizyme/ODC ratio in female mouse kidney was 24 times as high as that in males. A similar relationship between antizyme/ODC ratio and decay rate of ODC activity was confirmed by testosterone treatment, which is known to prolong the half-life of renal ODC (Seely *et al.*, 1982; Isomaa *et al.*, 1983; Loeb *et al.*, 1984). These results were consistent with the previous observation that the relative amount of ODC-antizyme complex correlates with the degradation rate of ODC, suggesting a key role of renal antizyme in ODC degradation. Finally, we confirmed that pretreatment of testosterone-treated mice with putrescine increased the antizyme/ODC ratio and accelerated ODC decay in the presence of cycloheximide (result not shown).

The mechanism whereby antizyme participates in degradation of ODC is obscure. In order to explain the reason why small amounts of antizyme can mediate the

decay of larger amounts of ODC after cycloheximide treatment without supply by synthesis *de novo*, we previously proposed (Hayashi *et al.*, 1985), as Seely & Pegg (1983*a*) had also suggested, that antizyme recycles during the process of ODC decay in a manner similar to the ubiquitin-dependent proteolysis in reticulocytes (Hershko & Ciechanover, 1982). Development of a cell-free system for ODC degradation is required to verify the function of antizyme in ODC degradation.

Finally, it is unknown which of putrescine, spermidine or spermine induces antizyme under physiological conditions. Brosnan *et al.* (1983) found that antizyme was induced in response to endogenous putrescine in mammary gland of lactating rats. On the other hand, Mitchell *et al.* (1985) showed that addition of spermidine, but not putrescine, caused a rapid loss of ODC activity in HTC cells treated with cyclohexylamine, an inhibitor of spermidine synthase. They suggested an essential involvement of a protein in the rapid loss of ODC, although they did not identify it as antizyme. The present results shown in Tables 3 and 4 suggested indirectly a contribution of spermidine and/or spermine to antizyme induction, since cellular contents of spermidine and spermine were the same in males and females, and the sexual difference in the amount of antizyme could be attributed to the difference in the degradation rate, but not in the synthesis rate, of antizyme.

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