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STUDIES ON ORNITHINE DECARBOXYLASE FROM THE LIVER OF THIOACETAMIDE-TREATED RATS

PURIFICATION AND SOME PROPERTIES

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SUMMARY

Ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) was purified about 5400-fold from the soluble fraction of liver from thioacetamide-treated rats. The purified enzyme showed only a single protein band on polyacrylamide gel electrophoresis. The molecular weight of the enzyme was estimated to be approx. 100 000. Its isoelectric point was found to be at pH 4.1 and its optimal pH around 7.0. The K_m value for L-ornithine was $2 \cdot 10^{-4}$ M at pH 7.0.

The enzyme required thiol compounds for maximal activity and was inhibited by inhibitors of pyridoxal enzymes, such as L-canaline and isonicotinic acid hydrazide. Among the various amino acids and amines tested, putrescine and D-ornithine caused a weak inhibition, while other compounds had little effect.

The properties of ornithine decarboxylase from the liver of thioacetamide-treated rats were compared with those of enzyme from regenerating rat liver.

INTRODUCTION

Recently, much attention has been focused on the physiological function of the polyamines, spermidine and spermine, in relation to nucleic acid metabolism, since their concentrations are highest in rapidly growing tissues including regenerating liver, chick embryos and certain tumors[1–10]. The level of spermidine is also very high in tissues with high rates of RNA and protein synthesis, such as bone marrow, pancreas, prostate and lactating mammary gland[6,11,12].

The synthesis of polyamines involves the decarboxylation of ornithine by ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) to putrescine, which is then linked to a propylamine moiety of decarboxylated *S*-adenosylmethionine to form spermidine. Ornithine decarboxylase is believed to be rate-limiting in spermidine

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synthesis, because its activity is greatly elevated under conditions that cause accumulation of polyamines[13,14] and the reaction product, putrescine, markedly stimulates the decarboxylation of *S*-adenosylmethionine[15–17]. Thus, in rat liver the activity of this enzyme is greatly enhanced after partial hepatectomy[5,13,18–23]. In addition, it has been shown that the administration of growth hormone[7,20,23–29] or thioacetamide[7,19,30] also results in a large augmentation of rat liver ornithine decarboxylase.

Recently, Jänne and Williams-Ashman[31] purified ornithine decarboxylase about 300-fold from rat ventral prostate and studied some of its properties. However, this enzyme, and especially the enzyme in liver, has not been purified extensively. This paper describes the exhaustive purification of ornithine decarboxylase from the liver of thioacetamide-treated rats and a comparison of some of its properties with those of the enzyme from regenerating rat liver.

MATERIALS AND METHODS

Treatment of animals

Thioacetamide treatment. Adult Sprague–Dawley rats, weighing over 300 g, were used. The animals were given an intraperitoneal injection of 150 mg/kg body weight of thioacetamide in saline solution. 20 h later they were sacrificed by decapitation and the livers were excised.

Partial hepatectomy. Male rats of the Sprague–Dawley strain, weighing 150–200 g, were used. Partial hepatectomy was performed by the method of Higgins and Anderson[32]. 18 h after the operation, the animals were killed by decapitation and the livers were removed.

Fractionation of subcellular components of the liver

Mitochondria, microsomes and a soluble fraction were prepared by the procedure of Hogeboom[33], except that the 0.25 M sucrose solution contained 10 mM sodium phosphate buffer (pH 7.2), 0.2 mM pyridoxal phosphate and 5 mM dithiothreitol to prevent inactivation of ornithine decarboxylase, and that microsomes were precipitated by centrifugation at 105 000 × *g* for 60 min.

Nuclei were isolated by the method of Chauveau *et al.*[34] using 2.2 M sucrose containing 10 mM sodium phosphate buffer (pH 7.0) for homogenization.

Nuclei and mitochondria were then disrupted by homogenization withglass powder in 10 mM sodium phosphate buffer (pH 7.0), containing 0.2 mM pyridoxal phosphate and 5 mM dithiothreitol, using a glass homogenizer and the homogenate was centrifuged at 10 000 × *g* for 20 min.

Assay for ornithine decarboxylase activity

The activity of ornithine decarboxylase was determined by measuring the liberation of $^{14}CO_2$ from L-[1-^{14}C]ornithine by a modification of the method of Russell and Snyder[5]. The incubation was carried out in a Warburg flask with two sidearms and fitted with a serum bottle stopper. The standard assay mixture in the main compartment contained 25 μmoles of sodium phosphate buffer (pH 7.0), 0.1 μmole of pyridoxal phosphate, 5 μmoles of dithiothreitol and enzyme (0.05–2 units) in a total volume of 0.8 ml. One sidearm contained 0.173 μmole of DL-[1-^{14}C]ornithine (4.63 mCi/mmole) and 1.913 μmole of L-ornithine as substrate in a volume of 0.2 ml, and

the other contained 0.3 ml of 4 M citric acid. The center well contained 0.2 ml of 1 M hyamine hydroxide. After preincubation for 2 min at 37 °C, the reaction was started by tipping in the substrate. After shaking for 30 min, the reaction was stopped by addition of citric acid from the second sidearm. The reaction mixture was incubated for an additional 60 min to trap the $^{14}CO_2$ evolved. The hyamine solution was then transferred to a counting vial with 10 ml of scintillator–toluene solution. Radioactivities were measured in a Packard Tri-Carb liquid scintillation spectrometer. Values were corrected by subtracting the value of mixture without enzyme.

One unit of enzyme is defined as the amount of enzyme forming 1 nmole of CO_2 from L-ornithine per min. The specific activity is defined as the number of units per mg protein.

Determination of protein

Protein concentrations were determined by the procedure of Lowry *et al.*[35] with bovine serum albumin as standard. When the samples contained thiols, such as 2-mercaptoethanol or dithiothreitol, the values obtained were corrected by subtracting the values of blanks containing these thiols.

Disc electrophoresis

The purity of the enzyme preparation was checked by disc electrophoresis. Routine runs were performed by the method of Williams and Reisfeld[36] in barbital buffer (pH 8.3), using an acrylamide concentration of 7.5% in a vertical electrophoresis apparatus (M and S Intruments Trading Co., Inc., Osaka, Japan). The protein samples contained about 15 μg of enzyme at Step 7 from the liver of thioacetamide-treated rats. Electrophoresis was carried out at 3 mA per tube for 90 min. After development, the gel was stained with Amido black and excess stain was removed with 7% acetic acid.

Isoelectric focusing

Isoelectric focusing was conducted by the method of Haglund[37], using an electrolysis column of 110 ml volume. Materials were separated on a gradient of pH 4 to 6 applying 1.5 mA at 500 V for 39 h. After electrolysis, the contents of the column were collected in 2-ml fractions in test tubes for analysis.

Concentration of enzyme solution

After each chromatographic step during enzyme purification, active fractions were pooled and concentrated in an Amicon ultrafiltration unit consisting of a Dia-Flo ultrafiltration membrane UM-10 and a Dia-Flo Model 50 or Model 402 ultrafiltration cell at 4 °C. Pressure was maintained by introducing nitrogen gas during concentration of the enzyme. Recovery was quantitative with respect to enzyme activity.

Materials

DL-[1-^{14}C]Ornithine·HCl was purchased from New England Nuclear Corporation, Boston, Mass., U.S.A., and D-ornithine and L-canaline were from Sigma Chemical Co., St. Louis, Mo., U.S.A. Dithiothreitol was obtained from Calbiochem, Los Angeles, Calif., U.S.A. A kit of molecular weight markers was obtained from Schwarz/Mann, Orangeburg, N.Y., U.S.A. 2-Mercaptoethanol was purified by distillation. Electro-

focusing equipment and reagents were purchased from LKB AB, Stockholm-Bromma, Sweden. Various ornithine derivatives employed in the present study were kindly supplied by Dr M. Okada, Research Laboratories, Chugai Pharmaceutical Co., Ltd, Tokyo, Japan. Other materials used were standard commercial products.

RESULTS

Subcellular distribution of ornithine decarboxylase

First, the intracellular distribution of ornithine decarboxylase was examined with subcellular fractions isolated from the livers of thioacetamide-treated and hepatectomized rats. Table I shows that the distributions of ornithine decarboxylase between the subcellular fractions were similar in the two types of liver and about 90% of the enzyme activity was found in the soluble, cytoplasmic fraction. Very low activity was present in the microsomal fraction and there was no detectable activity in the mitochondria. The specific activity in the nuclear fraction ranged from one-fifth to one-third of that in the soluble fraction. Therefore, the soluble fraction was used for enzyme purification.

TABLE I

SUBCELLULAR DISTRIBUTION OF ORNITHINE DECARBOXYLASE IN THIOACETAMIDE-TREATED AND REGENERATING RAT LIVER

Fraction	*Ornithine decarboxylase activity*					
	*Thioacetamide-treated**			*Regenerating***		
	Units/mg protein	*Units/g liver****	*% Distribution*	*Units/mg protein*	*Units/g liver****	*% Distribution*
Nuclei	0.0060	0.126	6.2	0.0090	0.189	10.9
Mitochondria	0	0	0	0	0	0
Microsomes	0.0024	0.050	2.4	0.0025	0.053	3.1
Soluble fraction	0.0333	1.865	91.4	0.0266	1.490	86.0

* 18 h after intraperitoneal injection of 150 mg/kg body weight of thioacetamide.
** 18 h after partial hepatectomy.
*** The values were calculated from the specific activity of each fraction and the subcellular distribution of protein based on the data of Price *et al.*[43].

Purification of ornithine decarboxylase from liver of thioacetamide-treated rats

Step 1. Preparation of crude extract. Fresh livers (about 7 kg) of rats treated with thioacetamide were homogenized in 3 vol. of 0.25 M sucrose containing 10 mM sodium phosphate buffer (pH 7.0), 0.1 mM EDTA and 20 mM 2-mercaptoethanol using a glass homogenizer. The homogenate was centrifuged at 15 000 × *g* for 15 min and then at 105 000 × *g* for 45 min. The supernatant fluid (crude extract) was pooled. All operations, including Step 1, were conducted at 0–3 °C, unless otherwise mentioned.

Step 2. Acid treatment. The crude extract was adjusted to pH 4.6 by adding chilled 2 M acetic acid slowly with stirring. Then the mixture was immediately centrifuged at 15 000 × *g* for 10 min and the precipitate was homogenized with a Teflon homogenizer with 50 mM sodium phosphate buffer (pH 7.4), containing 0.1 mM

pyridoxal phosphate, 0.1 mM EDTA and 20 mM 2-mercaptoethanol, in a total of 0.1 of the volume of the original crude extract. The homogenate was dialyzed against 25 vol. of 10 mM sodium phosphate buffer (pH 7.0), containing 0.1 mM pyridoxal phosphate, 0.1 mM EDTA and 20 mM 2-mercaptoethanol, for 8 h with two changes of the outer fluid. The dialyzed solution was centrifuged at 15 000 × *g* for 10 min. The precipitate was washed with the same buffer and centrifuged, and the washing fluid was combined with the supernatant. All the subsequent steps were performed in the presence of 0.1 mM pyridoxal phosphate, 0.1 mM EDTA and 20 mM 2-mercaptoethanol, unless otherwise indicated.

Step 3. DEAE-cellulose column chromatography. Each run was carried out with about 5 g of protein. Enzyme from Step 2 was diluted with 10 mM sodium phosphate buffer (pH 7.5) to give 30 mg of protein per ml and then applied to a column of DEAE-cellulose (3.9 cm × 32 cm), equilibrated with 10 mM sodium phosphate buffer (pH 7.5). The column was washed with 400 ml of the same buffer and eluted with a continuous gradient of KCl in 10 mM sodium phosphate buffer (pH 7.5), obtained by placing 20 mM buffer (900 ml) in the mixing chamber and 400 mM buffer (900 ml) in the reservoir. The eluate was collected in 11-ml fractions at a flow rate of 3.5 ml/min. Fractions with a specific activity of over 1.45 were collected and concentrated by ultrafiltration.

Step 4. First gel filtration. Concentrated enzyme from Step 3 was diluted with 5 mM sodium phosphate buffer (pH 7.0) to a protein concentration of 10 mg/ml. This solution was mixed with saturated $(NH_4)_2SO_4$ solution containing 5 mM sodium phosphate buffer (pH 7.0). The precipitate formed between 35 and 55% saturation was collected by centrifugation and dissolved in a small volume of 5 mM sodium phosphate buffer (pH 7.0). This solution was immediately applied to a column of Sephadex G-150 (2.1 cm × 90 cm), equilibrated with 5 mM sodium phosphate buffer (pH 7.0). The column was eluted with the same buffer and 4-ml fractions were collected at a flow rate of about 15 ml/h. Fractions with a specific activity of over 9.0 were collected and concentrated by ultrafiltration.

Step 5. Second gel filtration. The above enzyme was diluted to 10 mg of protein per ml and again subjected to $(NH_4)_2SO_4$ fractionation in the same way as in Step 4. The precipitate formed between 40 and 50% saturation was collected by centrifugation and dissolved in a minimal volume of 5 mM sodium phosphate buffer (pH 7.0). This solution was then subjected to gel filtration on a column of Sephadex G-150 and eluted in a similar manner as in Step 4. Fractions with a specific activity of over 32.12 were collected and concentrated by ultrafiltration.

Step 6. Isoelectric precipitation. The concentrated enzyme from Step 5 was diluted with 5 mM sodium phosphate buffer (pH 7.0) to a protein concentration of 5 mg/ml. The diluted enzyme was then acidified to pH 4.3 by addition of chilled 50 mM acetic acid and the precipitate formed was removed by centrifugation. Further acetic acid was added to the resulting supernatant fluid to bring the pH to 4.1. The mixture was immediately centrifuged at 15 000 × *g* for 10 min and the precipitate was dissolved in a small volume of 5 mM sodium phosphate buffer (pH 7.0).

Step 7. Zone centrifugation in a sucrose gradient. Enzyme from Step 6 (0.23 ml) was layered on a linear gradient (4.5 ml) of 5 to 18% sucrose in 5 mM sodium phosphate buffer (pH 7.0) and centrifuged at 100 000 × *g* in a rotor equipped with three swinging buckets. After centrifugation for 18 h at 3 °C, fractions of 2 drops (0.11 ml)

TABLE II

PURIFICATION OF ORNITHINE DECARBOXYLASE FROM THIOACETAMIDE-TREATED RAT LIVER

Step	*Protein (mg)*	*Total activity (units)*	*Specific activity*	*Recovery (%)*	*Relative activity*
1. Crude extract	432 652	15 368	0.036	100	1
2. Acid treatment (pH 4.6)	22 355	11 642	0.52	75.8	14.4
3. DEAE-cellulose column chromatography	5 047	8 091	1.60	52.6	44.4
4. First gel filtration on Sephadex G-150	487.3	4 928	10.1	32.1	280.6
5. Second gel filtration on Sephadex G-150	32.2	1 435	44.6	9.3	1238.9
6. Isoelectric precipitation (pH 4.1)	12.1	716	59.2	4.7	1644.4
7. Sucrose density gradient	1.06	205	193.4	1.3	5372.2

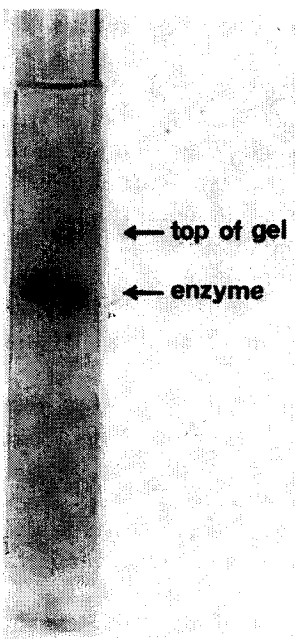


Fig. 1. Polyacrylamide gel electrophoresis of purified ornithine decarboxylase from liver of thioacetamide-treated rats. Electrophoresis was carried out with 15 μg of enzyme at Step 7. Conditions were as described in the text.

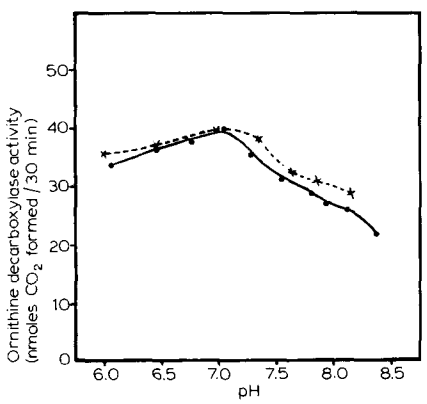
were collected by puncturing the bottom of the tube. Fractions with a constant specific activity of about 193 were pooled. The purification of the enzyme is summarized in Table II.

Enzyme at Step 7 was analyzed by polyacrylamide gel electrophoresis. As shown in Fig. 1, the preparation gave only a single band on the electrophoregram.

Properties of ornithine decarboxylase from thioacetamide-treated rat liver

Effect of pH. The pH–activity curves of the enzyme obtained using sodium phosphate and glycylglycine buffers at concentrations of 30 mM are shown in Fig. 2. In both buffers activity was maximal at pH 7.0. This pH value is lower than that of pH 7.4 obtained with the enzyme from regenerating rat liver by Raina and Jänne[38], and very close to that of the prostatic enzyme (pH 7.0) reported by Jänne and Williams-Ashman[31]. The latter investigators reported that the activity of a partially-purified preparation from rat prostate in 100 mM sodium phosphate (pH 7.2) was much less than in glycylglycine buffer. However, this was not the case with the liver enzyme which showed similar activities at the optimal pH in these two buffers at concentrations of 30 mM.

Isoelectric point. Isoelectrofocusing of enzyme at Step 4 was conducted with ampholines in a pH range from 4 to 6. As shown in Fig. 3, the isoelectric point of the enzyme protein was found at pH 4.1.



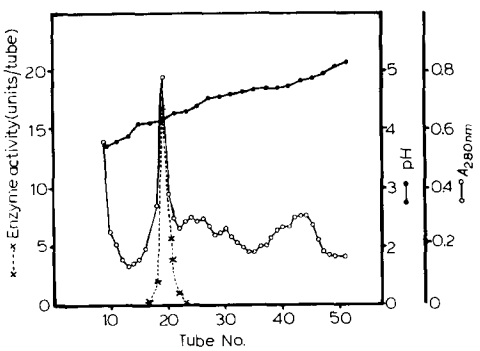


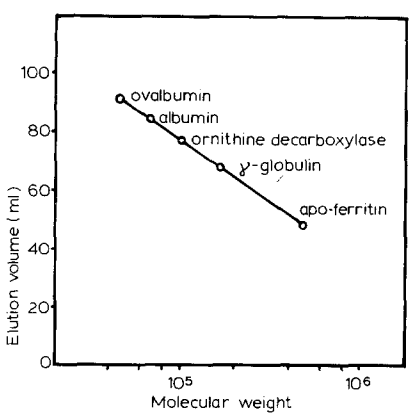
Fig. 2. Effect of pH on ornithine decarboxylase activity. Enzyme at Step 6 (23 μg of protein) was incubated under standard assay conditions in 30 mM sodium phosphate buffers (●—●) or 30 mM glycylglycine buffers (×---×) of various pH values. The pH values indicated were determined experimentally at the end of the incubation.

Fig. 3. Isoelectric focusing of ornithine decarboxylase from liver of thioacetamide-treated rats. 12 mg of enzyme at Step 4 (spec. act. 10.1) were used. Other conditions were as described in the text.

Molecular weight. The molecular weight of the enzyme was estimated by gel filtration on a column of Sephadex G-150 following the method of Andrews[39] in 5 mM sodium phosphate buffer (pH 7.0) containing 0.1 mM pyridoxal phosphate, 0.1 mM EDTA and 5 mM dithiothreitol. When the elution volume of the enzyme was correlated with the elution volumes and molecular weights of proteins of known molecular weights, a value of about 100 000 was obtained as the molecular weight of ornithine decarboxylase from liver of thioacetamide-treated rats, though it is only an approximation (Fig. 4).

K_m value for L-*ornithine.* The K_m value for L-ornithine at pH 7.0 was calculated to be $2 \cdot 10^{-4}$ M from the data presented in Fig. 5. This value is in good agreement with that obtained with enzyme from regenerating rat liver by Raina and Jänne[38].

Stability. The purified enzyme was labile on storage and retained only about



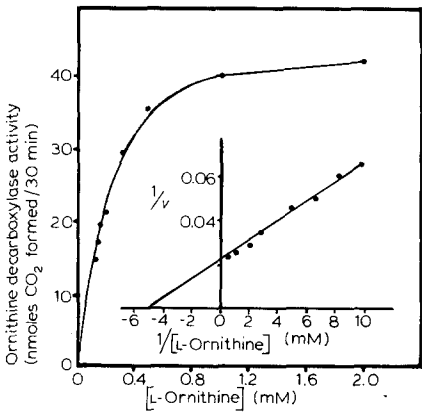


Fig. 4. Determination of molecular weight of ornithine decarboxylase by gel filtration on Sephadex G-150. The column (1.5 cm × 90 cm) was equilibrated with 5 mM sodium phosphate buffer (pH 7.0), containing 0.1 mM pyridoxal phosphate, 0.1 mM EDTA and 5 mM dithiothreitol. Enzyme at Step 6 (2 mg in 1 ml) was applied to the column. The standard curve indicates the relationship between the elution volume and molecular weight. The protein concentration of all standards was about 2 mg in 1 ml.

Fig. 5. Effect of L-ornithine concentration on ornithine decarboxylase activity. Enzyme activity was measured with enzyme at Step 6 (24 μg of protein) under standard assay conditions with various concentrations of L-ornithine. The amount of DL-[1-^{14}C]ornithine was kept constant, but the specific radioactivity was varied by addition of suitable quantities of L-ornithine.

TABLE III

EFFECT OF THIOLS ON ORNITHINE DECARBOXYLASE ACTIVITY

25 μg (10 μl) of enzyme at Step 5 (spec. act. 43.0) in 10 mM sodium phosphate buffer (pH 7.0) containing 0.1 mM pyridoxal phosphate, 0.1 mM EDTA and 20 mM 2-mercaptoethanol were added to the standard assay mixture in a total volume of 1 ml. Therefore, the reaction mixture without added thiol contained a final concentration of 0.2 mM 2-mercaptoethanol. After preincubation of the enzyme at 37 °C for 15 min in the presence of the various concentrations of thiol compounds indicated, the reaction was started by addition of labeled ornithine.

Addition	*Concentration (mM)*	*Ornithine decarboxylase activity*	
		Units/mg protein	*%*
None	—	8.63	100
2-Mercaptoethanol	5	18.2	211
	10	23.1	268
	50	23.8	276
Dithiothreitol	1	32.1	372
	5	43.0	498
	10	40.7	472

one-fifth of its original activity after 24 h, when stored in sodium phosphate buffer (pH 7.0) at 2 °C without addition of thiols. This inactivation was prevented by addition of thiols, such as 2-mercaptoethanol, GSH or dithiothreitol, the last compound being the most effective. When the enzyme was kept at pH 7.0 in the frozen state at −20 °C in the presence of 5 mM dithiothreitol, no appreciable loss of activity was observed for at least 1 week. However, repeated freezing and thawing resulted in progressive inactivation.

Effects of thiols. Table III shows the effects of thiols on the activity of ornithine decarboxylase in the assay system. Addition of 2-mercaptoethanol or dithiothreitol greatly enhanced the enzyme activity, the latter being much the more effective. The maximal activity was obtained with 5 mM dithiothreitol, the value being about 5-fold higher than that obtained without thiols.

To demonstrate the requirement of the enzyme for thiols more clearly, the following experiments were conducted. Enzyme, which had been stored in the frozen state for about 5 months in the presence of 20 mM 2-mercaptoethanol, 0.1 mM pyridoxal phosphate and 10 mM sodium phosphate buffer (pH 7.0), was dialyzed against 200 vol. of 5 mM sodium phosphate buffer (pH 7.0), containing no thiols, for 5 h with two changes of the outer fluid. Then the enzyme activity was measured with or without preincubation in the presence of added thiols. As shown in Table IV, enzyme activity was very low when assayed in the absence of added thiols. However, it was greatly increased by addition of thiols, such as 2-mercaptoethanol or dithiothreitol, and preincubation of the enzyme with thiols for 15 min at 37 °C further enhanced this effect of thiols. The degree of reactivation was dependent on the concentration of thiols added and dithiothreitol was again superior to 2-mercaptoethanol in this respect. Maximal activity was obtained with dithiothreitol at concentrations of 5–10 mM.

TABLE IV

REACTIVATION OF ORNITHINE DECARBOXYLASE BY THIOLS

Enzyme at Step 4 (10.1 units/mg protein, 14 mg of protein/ml) was stored in a frozen state at −20 °C in the presence of 10 mM sodium phosphate buffer (pH 7.0), 0.1 mM pyridoxal phosphate 0.1 mM EDTA and 20 mM 2-mercaptoethanol. After storage for about 5 months, the enzyme was dialyzed against 200 vol. of the same buffer containing no thiols for 5 h with two changes of the outer fluid. The dialyzed enzyme was centrifuged at 10 000 × *g* for 10 min and the resulting supernatant fluid was used as enzyme. Preincubation was carried out for 15 min at 37 °C with the thiols indicated.

Thiol added	*Concentration (mM)*	*Ornithine decarboxylase activity*			
		Without preincubation		*With preincubation*	
		Units/mg protein	*Activity recovered (%)*	*Units/mg protein*	*Activity recovered (%)*
None	—	0.008	0.1	0	0
2-Mercaptoethanol	5	0.177	1.7	0.674	6.7
	10	0.637	6.3	1.774	17.6
	20	1.588	15.7	2.499	24.7
Dithiothreitol	1	1.646	16.3	3.911	38.7
	5	2.471	24.5	4.847	48.0
	10	3.153	31.2	5.480	54.3

Effects of inhibitors of pyridoxal enzymes. L-Canaline, a structural analogue of L-ornithine, which is known to inhibit pyridoxal enzymes[40], strongly inhibited the reaction. The inhibition was about 50% at a concentration of 0.01 mM even in the presence of 0.1 mM pyridoxal phosphate (Table V). Isonicotinic acid hydrazide also inhibited the enzyme, although less than L-canaline and about 50% inhibition was observed at

TABLE V

EFFECT OF L-CANALINE AND ISONICOTINIC ACID HYDRAZIDE ON ORNITHINE DECARBOXYLASE ACTIVITY

81.6 μg of enzyme at Step 4 were used. The enzyme was preincubated with L-canaline or isonicotinic acid hydrazide for 2 min at 37 °C and then the reaction was started by adding labeled ornithine.

Addition	*Concentration (mM)*	*Ornithine decarboxylase activity**	*Inhibition (%)*
None	—	24.8	0
L-Canaline	0.01	12.4	48
	0.05	2.8	88
	0.1	2.1	91
Isonicotinic acid hydrazide	0.1	20.2	15
	0.5	17.8	25
	1.0	11.7	51

* Enzyme activity is expressed as nmoles of $^{14}CO_2$ evolved per 30 min.

TABLE VI

EFFECTS OF VARIOUS AMINES AND D-ORNITHINE ON ORNITHINE DECARBOXYLASE ACTIVITY

87.1 μg of enzyme at Step 4 were used. The enzyme was preincubated with various amines or D-ornithine for 2 min at 37 °C before starting the reaction.

Addition	*Concentration (mM)*	*Ornithine decarboxylase activity**	*Inhibition (%)*
None	—	26.4	0
Putrescine	5	22.6	14.5
	10	19.8	24.7
Spermidine	5	25.6	3.0
	10	23.3	11.8
Spermine	10	24.6	6.5
Cadaverine	10	25.4	4.0
Histamine	10	22.2	15.7
D-Ornithine	5	23.9	9.4
	10	19.9	24.4

* Enzyme activity is expressed as nmoles of $^{14}CO_2$ evolved per 30 min.

a concentration of 1 mM. These results are in agreement with the fact that pyridoxal phosphate is the cofactor of this enzyme.

Effects of other compounds. Putrescine, one of the reaction products, showed a weak inhibitory effect. It inhibited the reaction by about 25% at a concentration of 10 mM (Table VI) and had virtually no effect at a concentration of 1 mM. The other amines tested, such as spermidine, spermine, cadaverine and histamine, were less inhibitory than putrescine.

D-Ornithine caused about 10% and 25% inhibition at concentrations of 5 mM and 10 mM, respectively. This weak inhibitory action of D-ornithine shows that DL-[1-^{14}C]ornithine can be used as a substrate, since the assay mixture contained only a

trace of labeled D-ornithine and a large excess of unlabeled L-ornithine.

None of the other amino acids or derivatives of ornithine tested had any effect at a concentration of 10 mM. The compounds tested were L-lysine, L-arginine, L-histidine, L-citrulline, L-glutamine, α- or δ-*N*-acetylornithine, α- or δ-*N*-benzoyl-L-ornithine, α- or δ-ornithylornithine, α-aminoadipic acid and α-aminovaleric acid.

Comparison of ornithine decarboxylase from regenerating rat liver with that from the liver of thioacetamide-treated rats

After partial hepatectomy the activity of ornithine decarboxylase in the liver increased greatly[5,13,18–23] and most of the induced activity was found in the soluble fraction (Table I). Using exactly the same procedure as that shown in Table II for liver from thioacetamide-treated rats, the enzyme was purified about 630-fold at Step 5 from 690 g of livers obtained 18 h after partial hepatectomy. The specific activity of the crude extract was 0.054, which was slightly higher than that of liver from thioacetamide-treated rats. Using this partially-purified preparation (Step 5), the properties of the enzyme from regenerating liver were compared with those of enzyme from the liver of thioacetamide-treated rats. All the properties of the two enzymes examined were the same. Namely, their optimal pH values, isoelectric points, K_m values for L-ornithine, requirements for thiols and molecular weights were the same.

DISCUSSION

It has been proposed that ornithine decarboxylase plays a regulatory role in the synthesis of polyamines, which may be important in rapidly growing tissues, and that a striking increase in enzyme activity results in initiation of rapid growth. However, there are only a few reports of the purification and properties of this enzyme. Raina and Jänne[38] studied some properties of this enzyme using a preparation fractionated with ammonium sulfate from regenerating rat liver and Friedman *et al.*[41] achieved about 175-fold purification over a crude extract from the same source. Recently, Jänne and Williams-Ashman[31] purified enzyme about 300-fold from rat ventral prostate and described some of its properties, especially the effects of thiol compounds on it. In this work, we purified ornithine decarboxylase from liver of thioacetamide-treated rats and the purified preparation appeared as a single protein on polyacrylamide gel electrophoresis. The specific activity of the purified preparation (193.4 nmoles of CO_2 evolved from ornithine per mg protein per min) was about 5400-fold that of the crude extract. The value indicates that there is a very low content of this enzyme in rat liver even after induction with thioacetamide. The result also shows that at least 3600-fold purification is necessary to obtain a homogeneous enzyme from regenerating rat liver, though the specific activity of the crude extract from this source is a little higher than that from liver of thioacetamide-treated rats (0.054 *vs* 0.036). Thus, the enzyme preparation obtained by Friedman *et al.*[41] from regenerating rat liver was probably highly contaminated even after 175-fold purification, although these workers stated that their preparation exhibited a single major band on acrylamide gel electrophoresis. Likewise, the specific activity of the 300-fold purified enzyme of Jänne and Williams-Ashman[31] from rat ventral prostate is calculated to be about 10 nmoles of CO_2 evolved from ornithine per mg protein per min, and this value is only about one-twentieth of that of our purified preparation.

The properties of ornithine decarboxylase from liver of thioacetamide-treated rats are quite similar to those of the enzyme from rat ventral prostate reported by Jänne and Williams-Ashman[31]. These properties include the optimal pH, stability on storage, K_m value for L-ornithine and differential effects of 2-mercaptoethanol and dithiothreitol on enzyme activity. Thiol compounds greatly stimulate the enzyme activity and also considerably reactivate decarboxylase, which has become inactivated on storage. Both effects are greater with dithiothreitol than with 2-mercaptoethanol. By analogy with the results of Jänne and Williams-Ashman[22,31] on the rat prostatic enzyme, it seems likely that these effects are closely related to the changes in physical state of the enzyme molecule, but this was not investigated in the present study.

Pegg and Williams-Ashman[42] observed a significant inhibition (K_i = 1.2 mM) of the rat prostatic enzyme by putrescine, one of the reaction products. On the other hand, Raina and Jänne[38] observed only about 20% inhibition at a concentration of 20 mM with a crude preparation from regenerating rat liver. In the present work, the purified enzyme from liver of thioacetamide-treated rats was only inhibited 25% by 10 mM putrescine while spermidine and spermine were less inhibitory. Therefore, these polyamines appear to be of little physiological significance as regulators of ornithine decarboxylase in rat liver. Further studies on the immunochemical and physical properties of the enzyme are now in progress.

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