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PROPERTIES OF ORNITHINE AMINOTRANSFERASE FROM RAT LIVER, KIDNEY AND SMALL INTESTINE

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SUMMARY

1. A method for purification and crystallization of ornithine aminotransferase (L-ornithine:2-oxoacid aminotransferase, EC 2.6.1.13) from rat kidney is described.

2. Various properties of the enzymes from liver, kidney and small intestine were similar, including their ultracentrifugal and electrophoretic behavior, absorption spectra, pH optima, K_m values for ornithine and α-ketoglutarate, K_m value for pyridoxal phosphate and specificities for amino acceptors from ornithine.

3. The precipitation lines of the three enzymes against the antisera of kidney and liver ornithine amino transferase all fused with each other on Ouchterlony double diffusion plates.

4. From these results, the ornithine transaminase enzymes of liver, kidney and small intestine seem to be the same protein.

5. Only the activity of the liver enzyme was elevated *in vivo* by high protein diets. Injection of estradiol elevated only the activity of the kidney enzyme, and this was due to increase in ornithine aminotransferase protein estimated by immunoanalysis. The difference in the activities of kidney ornithine aminotransferase in male and female rats was due to the different contents of ornithine aminotransferase protein in their kidneys. The activity of ornithine aminotransferase in the small intestine did not change on these treatments.

INTRODUCTION

Previously we reported the purification of rat liver ornithine aminotransferase (L-ornithine:2-oxoacid aminotransferase, EC 2.6.1.13) to a single component and some of its properties[1]. In 1968 we crystallized this enzyme and reported some of its physicochemical properties[2]. PERAINO *et al.*[3] reported further details of its properties. Ornithine aminotransferase is widely distributed in various organs, and the kidney, small intestine and liver all have high activity[4]. However, there are no reports on chemical

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differences in the protein of ornithine aminotransferase in different organs. VOLPE *et al.*[5] reported that the metabolic role of ornithine aminotransferase in liver and kidney differed, the liver enzyme contributing the arginine synthesis, unlike the kidney enzyme. HERZFELD AND KNOX[4] reported that injection of estradiol increased the ornithine aminotransferase activity in the kidney but not in the liver. Recently, HERZFELD AND GREENGARD[6] presented detailed evidence that hormonal regulation of ornithine aminotransferase differed in liver and kidney. These reports suggest that isoenzymes of ornithine aminotransferase exist, but there is no report on differences in the enzyme protein using purified enzymes. We crystallized the ornithine aminotransferase from kidney; and we purified the enzyme from the small intestine to a single component. To study the organ specificity of ornithine aminotransferase we compared the properties of the enzymes from liver, kidney and small intestine at a molecular level, and the results are described in this paper.

MATERIALS AND METHODS

Unless otherwise indicated, male Wistar strain rats weighing 150–200 g were used. Enzyme activity was assayed as reported previously[2]. The molar extinction coefficient[7] of $2.71 \cdot 10^3$ was used to calculate the amount of pyrroline-5-carboxylate formed. The enzyme activity is expressed as units (μmoles of pyrroline-5-carboxylate per h at 37°) per ml of reaction mixture. Specific activity (S.A.) is expressed as units per mg protein.

Protein in crude extracts was measured by the biuret method with bovine serum albumin as standard[8]. With purified enzyme, protein was determined spectrophotometrically from the absorptions at 280 and 260 nm.

Electrophoresis was carried out on a cellulose acetate membrane (Carl Schleicher and Schüll) in 0.05 M potassium phosphate buffer, and membranes were stained with Ponceau 3R. For kinetic experiments, the apoenzyme was obtained by $(NH_4)_2SO_4$ precipitation at pH 6.0.

Ultracentrifugation was carried out in a Hitachi analytical ultracentrifuge, Type I. Sedimentation velocity was measured on centrifugation at 51 200 rev./min using a single cell.

Antiserum of ornithine aminotransferase was prepared as described previously[2]. Ouchterlony gel double diffusion analysis was performed as described by CLAUSEN *et al.*[9].

RESULTS

Purification of enzyme

Kidney ornithine aminotransferase. A sample of 55 g of rat kidney was homogenized in 5–6 vol. of ice-cold water containing 50 μg of pyridoxal phosphate per ml using a Potter–Elvehjem type Teflon homogenizer. Unless otherwise indicated, all subsequent steps were carried out at about 0°. The homogenate was then subjected to ultrasonic oscillation in a Kubota Sonicator (KMS-100) at 10 kcycles/sec for 2 min and centrifuged in a Hitachi ultracentrifuge, Type 18p at 15 000 rev./min for 20 min. The supernatant fluid was decanted, and potassium phosphate buffer (pH 6.0) and α-ketoglutarate were added to final concentrations of 0.05 M and 5 mM, respectively.

TABLE I

PURIFICATION OF KIDNEY ORNITHINE AMINOTRANSFERASE

Preparation	*Enzyme activity (units/ml)*	*Protein (mg/ml)*	*Total vol. (ml)*	*Total protein (mg)*	*Specific activity (units/mg)*	*Total activity (units)*	*Yield (%)*
Crude homogenate	32.3	24.0	300.0	7200	1.4	9690	100
Sonic extract	32.3	17.0	280.0	4760	1.9	9044	93.3
60° supernatant	20.4	4.0	285.0	1140	5.1	5814	58
Concn. solution in collodion bag	3720	7.8	1.5	12	477	5580	57
Crystallized enzyme	1387	1.5	4.0	6	924	5548	57

This solution was then heated in portions at 60° for 1 min by immersion in a water-bath at 75°, and cooled by immersion in ice-water. Heat-denatured protein was removed by centrifugation for 10 min at 12 000 rev./min. Solid $(NH_4)_2SO_4$ was added to the supernatant with stirring to a concentration of 50% (w/v). After further stirring for 10 min the mixture was centrifuged for 10 min at 12 000 rev./min, and the supernatant was discarded. The precipitate was suspended in 10 ml of 0.2 M potassium phosphate buffer (pH 8.0) and centrifuged for 10 min at 12 000 rev./min. The enzyme remained in the precipitate. The supernatant solution was discarded, and the precipitate was suspended in approx. 3 ml of ice-cold distilled water. This suspension was centrifuged for 30 min at 100 000 × *g*. The yellow supernatant solution contained the pure enzyme. This solution was concentrated to 1 ml in a collodion bag (Sartorius Membrane filter). The enzyme was crystallized in the same way as liver ornithine aminotransferase as reported previously[2] and as reported in more detail by PERAINO *et al.*[3]. Table I summarizes typical results obtained in the purification of kidney ornithine aminotransferase. Fig. 1 shows the appearance of crystals of this enzyme under a light microscope. The specific activity of the crystalline enzyme is about 920 units/mg protein. Recrystallized enzyme has the same specific activity as the

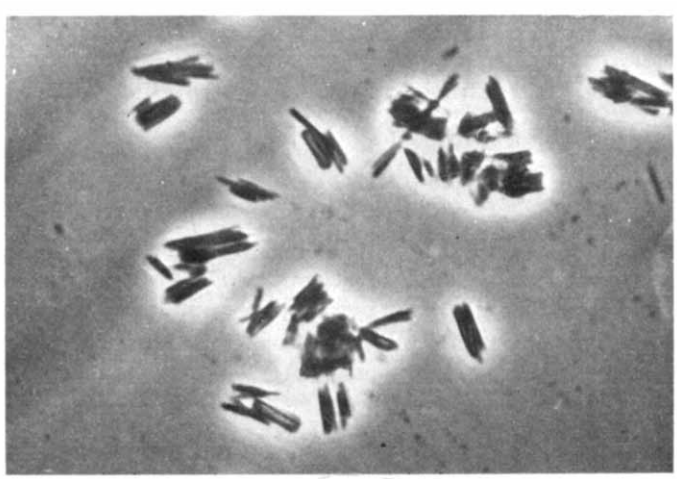


Fig. 1. Crystals of kidney ornithine aminotransferase.

enzyme after the first crystallization. This value is the same as that of the liver enzyme, and the crystals have a similar form[2].

Small intestine ornithine aminotransferase. The enzyme was purified by almost the same procedure for its solubilization. The enzyme was extracted from an acetone powder of small intestine because this tissue contains much lipid.

Electrophoretic mobilities

Fig. 2 shows the electrophoresis of the purified enzymes from liver, kidney and small intestine on a cellulose acetate membrane. The enzymes gave single bands at pH 6.0 and 8.0. These results indicate that the three enzymes have the same electrophoretic mobilities.

Absorbance spectrum

The spectrum of the kidney enzyme in 0.1 M potassium phosphate buffer (pH 8.0) has an absorption maximum at 420 nm due to the bound pyridoxal phosphate,

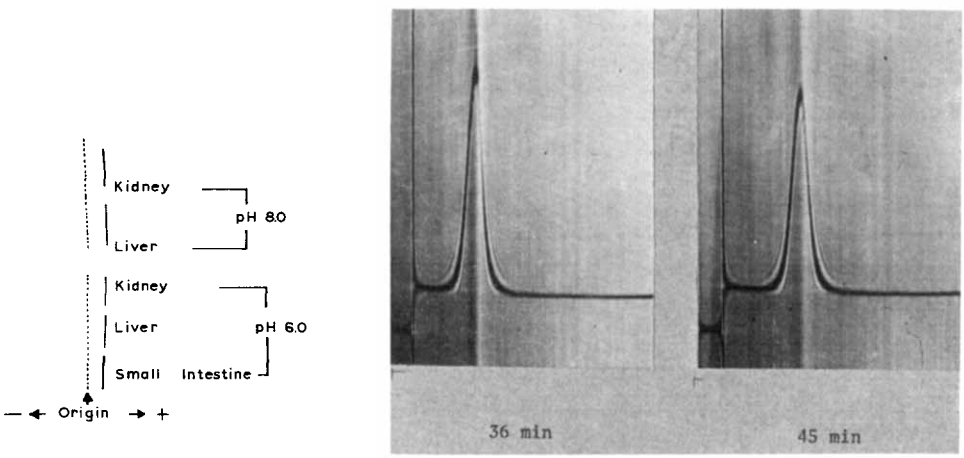


Fig. 2. Cellulose acetate membrane electrophoretograms of ornithine aminotransferase from liver, kidney and small intestine. Each enzyme was applied at the origin. Electrophoresis was performed at pH 8.0 and 6.0 in 0.05 M potassium phosphate buffer at 1 mV/cm for 50 min.

Fig. 3. Sedimentation pattern of kidney ornithine aminotransferase. The sedimentation is from left to right at a concentration of 14 mg of protein per ml in 0.1 M potassium phosphate buffer at 7°.

and this absorption spectrum is unchanged at pH values between 6.2 and 8.1. On addition of 0.1 M L-ornithine to the enzyme in the pyridoxal phosphate form a new peak appeared at 330 nm with a corresponding decrease in the absorption at 420 nm, indicating a conversion of the pyridoxal phosphate form to the pyridoxamine phosphate form. The $A_{280\ nm}/A_{420\ nm}$ ratio of enzyme in the pyridoxal phosphate form is about 8 in 0.1 M potassium phosphate buffer (pH 8.0). The absorption spectrum is the same as that of liver enzyme reported previously[2]. Both holoenzymes contain 2 molecules of pyridoxal phosphate per molecule[2].

TABLE II

INDEPENDENCE OF THE SEDIMENTATION CONSTANT OF KIDNEY ORNITHINE AMINOTRANSFERASE ON ENZYME CONCENTRATION

Values were measured in 0.1 M potassium phosphate buffer at 7°, and calculated for water at 20°.

Enzyme concn. (mg/ml)	$s_{20,w}$
1.75	9.916
3.5	8.87
7.0	10.01
14.0	10.04

Ultracentrifugal analysis

Fig. 3 shows the ultracentrifugal pattern of the kidney enzyme. The sedimentation constant of kidney ornithine aminotransferase obtained by extrapolation of the sedimentation velocity values observed at finite concentrations to zero concentration is 10.05 $s_{20,w}$ (Table II). The kidney enzyme has the same sedimentation velocity as the liver enzyme. This indicates that the two enzymes have the same molecular weight.

Kinetic and chemical properties

Fig. 4 shows the pH optima of three enzymes. The K_m values of these three enzymes for the substrates ornithine and α-ketoglutarate, and for the coenzyme pyridoxal phosphate, are shown in Table III. Table IV shows the specificities of amino acceptors for ornithine. These results indicate that the three enzymes have similar chemical properties.

Immunochemical analysis

On Ouchterlony double diffusion plates, the precipitation lines of the specific antibodies for highly purified liver and kidney enzymes with the purified kidney,

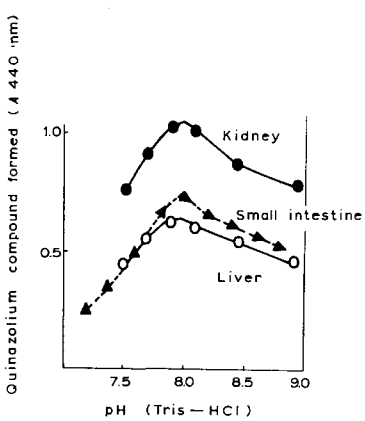


Fig. 4 pH–activity curves of ornithine aminotransferase from liver, kidney and small intestine in Tris–HCl buffer. The assay mixture contained, in 2 ml, 0.05 M of Tris–HCl buffer, 20 mM of ornithine, 10 mM of α-ketoglutarate, 5 μg of pyridoxal phosphate, and enzyme.

TABLE III

MICHAELIS CONSTANTS (K_m) OF ORNITHINE AMINOTRANSFERASE FROM LIVER, KIDNEY AND SMALL INTESTINE

The assay mixture for the evaluation of the K_m for ornithine contained, in 2 ml, 50 mM of potassium phosphate buffer (pH 8.0), 5 μg of pyridoxal phosphate, 10 mM of α-ketoglutarate and enzyme. The assay mixture for the evaluation of the K_m for α-ketoglutarate contained, in 2 ml, 50 mM of buffer, 5 μg of pyridoxal phosphate, 20 mM of ornithine and enzyme. For pyridoxal phosphate the mixture contained, in 2 ml, 10 mM of α-ketoglutarate, 20 mM of ornithine and enzyme. The enzyme was preincubated with pyridoxal phosphate for 30 min.

	Concentration (mM)		
	Ornithine	*α-Keto-glutarate*	*Pyridoxal phosphate*
Liver	0.56	0.91	$0.85 \cdot 10^{-3}$
Kidney	0.59	0.91	$0.83 \cdot 10^{-3}$
Small intestine	0.60	0.95	

TABLE IV

SPECIFICITIES OF VARIOUS AMINO ACCEPTORS FOR ORNITHINE WITH ORNITHINE AMINOTRANSFERASE FROM LIVER KIDNEY AND SMALL INTESTINE

The assay mixture contained, in 2 ml, 50 mM of potassium phosphate buffer, 20 mM of ornithine, 5 μg of pyridoxal phosphate, 10 mM of a keto-acid and enzyme. Activities were obtained by subtracting the product of the nonenzymic reaction. Values are the activities of each ketoacid as percentages of that of α-ketoglutarate.

	α-Ketoglutarate (%)	*Glyoxalate (%)*	*Pyruvate (%)*	*Oxaloacetate (%)*	*α-Ketobutyrate (%)*
Liver	100	20	8	2	1
Kidney	100	20	8	2	0
Small intestine	100	19	7	1	0

liver and small intestine enzymes fuse completely (Fig. 5). This indicates that the enzymes from kidney, liver and small intestine are indistinguishable immunochemically.

Effects of a high protein diet and estradiol on these three enzymes

The induction of the liver enzyme by a high protein diet has been reported in

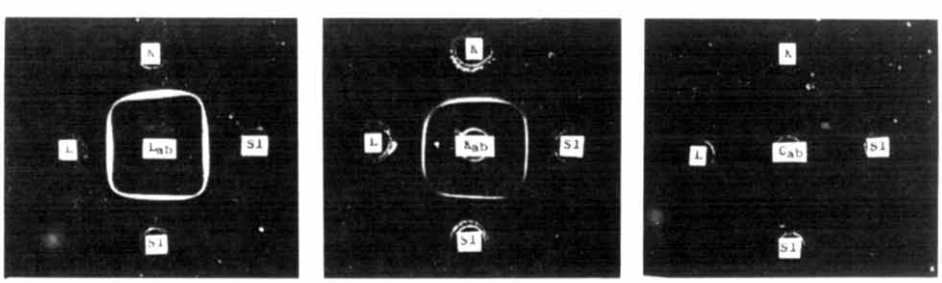
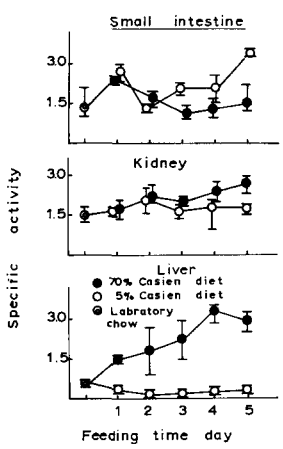


Fig. 5. Double immunodiffusion patterns of ornithine aminotransferase from liver, kidney and small intestine; K, enzyme from kidney; L, enzyme from liver; Sl, enzyme from small intestine; Lab, antiserum of liver enzyme ; Kab, antiserum of kidney; Cab, antiserum of adjuvant only.



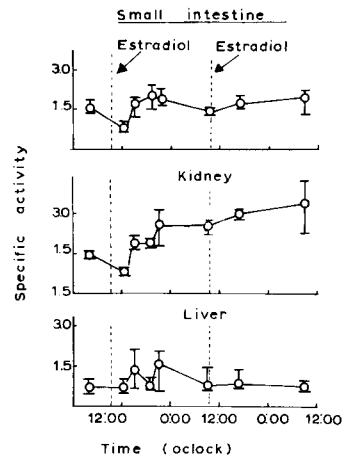


Fig. 6. Effect of high protein diets on the activities of ornithine aminotransferase in liver, kidney and small intestine. Diets containing casein, corn, vitamins, salt mixture and sucrose were administered to rats ad libitum. Each value is the mean of results on 3 animals.

Fig. 7. Effect of estradiol on the activities of ornithine aminotransferase in liver, kidney and small intestine. 200 μg of estradiol per 100 g body weight were injected intraperitoneally. Each value is the mean of results on 3 animals.

many papers[10–12]. Induction of the kidney enzyme by estradiol was reported by HERZFELD AND KNOX[4]. The changes in the enzyme activities in rat kidney, liver and small intestine caused by a high protein diet and injection of estradiol are shown in Figs. 6 and 7. The activity in small intestine does not change on either treatment. HERZFELD AND KNOX[4] reported that the ornithine aminotransferase activity in the kidney is higher in female than in male rats. The use of antiserum for the kidney

TABLE V

IMMUNOTITRATION OF MALE AND FEMALE RAT KIDNEY ORNITHINE AMINO-TRANSFERASE

Kidneys were homogenized with 5 vol. of 0.3 M KCl containing 5 μg/ml of pyridoxal phosphate, and the homogenate was subjected to ultrasonication at 10 kcycles/sec for 2 min. Then it was centrifuged at 100 000 × *g* for 60 min., and the supernatant was used for assay. The assay mixture contained, in 1 ml, 0.5 ml of supernatant, 0.2 ml of 0.2 M potassium phosphate buffer (pH 7.5) and 0.3 ml of antiserum of sufficient strength to precipitate the supernatant enzyme. The precipitate was washed with the same buffer. After centrifugation, the precipitate was suspended in the same buffer, and enzyme activity was assayed without loss of activity in the antigen-antibody reaction.

	Before antigen–antibody reaction		*After antigen–antibody reaction*
	Activity (units)	*Specific activity (units/mg)*	*Activity of precipitate (units)*
Male	15.5	0.63	12.3
	17.7	0.66	13.1
Female	22.7	0.84	17.7
	22.7	0.91	18.3

TABLE VI

IMMUNOTITRATION OF ORNITHINE AMINOTRANSFERASE ACTIVITY AFTER ESTRADIOL INJECTION
Estradiol (200 μg/100 g body weight per day) was injected subcutaneously into rats for 3 days. Animals were killed 14 h after the last injection. Control rats received injections of 0.9% NaCl. The enzyme preparation was obtained as described in Table V. Values for antiserum required are expressed as mg of antiserum protein needed to precipitate all the supernatant enzyme activity.

	Kidney			*Liver*		
	Activity (units)	*Specific activity (units/mg)*	*Antiserum required (mg)*	*Activity (units)*	*Specific activity (units/mg)*	*Antiserum required (mg)*
Control	40.5	3.0	3.2	14.2	0.6	2.4
	51.5	3.0	4.8	15.9	0.6	2.9
	54.5	3.0	5.8	13.6	0.6	2.4
After injection of estradiol	188.0	10.9	12.8	7.5	0.3	1.6
	91.5	5.6	9.0	6.5	0.3	1.6
	172.0	10.2	12.2	11.0	0.4	1.6

enzymes makes it clear (Table V) that this difference in activity is due to a difference in the quantity of enzyme. The female kidney contains about twice as much enzyme as male kidney. Similarly, Table VI shows that the elevation of the activity in kidney after injection of estradiol is due to an increase in the enzyme protein content.

DISCUSSION

The ornithine aminotransferase enzymes in liver, kidney and small intestine were compared on a molecular level. No significant difference between them was found. In other experiments, such as those on inhibition by branched-chain amino acids[1], the three enzymes had the same properties. Nevertheless the three enzymes show organ specific behavior in response to dietary and hormonal control, as already established. In the present work this was confirmed by further experiments on small intestine ornithine aminotransferase. Previously, we reported on the organ specific isozymes of glutaminase (E.C. 3.5.1.2) in rat liver, kidney and brain which show typical organ specificity. The glutaminase proteins located in these three organs are entirely different[13,14]. Although the ornithine aminotransferase enzymes in the three different organs are the same protein, their regulations differ.

What are the different metabolic roles of ornithine aminotransferase in the liver, kidney and small intestine? Many investigators have suggested that liver ornithine aminotransferase is involved in ornithine synthesis while the kidney enzyme is involved in glutamate or proline synthesis[4,5]. We have already reported that liver ornithine aminotransferase regulates the content of ornithine and thereby regulates the urea cycle[10,16]. In the kidney, ornithine aminotransferase may couple with glycine amidinotransferase and act in the metabolic regulation of proline and creatine formation.

Another important problem is what are the different mechanisms controlling the activities of ornithine aminotransferase in liver, kidney and small intestine? The enzymes in liver, kidney and small intestine probably differ slightly in primary structure, though the difference is too slight to detect by usual physicochemical and en-

zymic methods. If this is so, since the gene for each enzyme is different, the effect of hormones on the enzyme pattern in each organ may be different. Another possibility is that the genes for the enzymes and the enzyme proteins themselves in liver, kidney and small intestine are identical, and that estradiol and other effectors have different actions on the repression-derepression mechanisms in each organ, resulting in the organ specific behavior. Recently, DOYLE AND SCHIMKE[15], reported similar observations and speculations on the genetic regulation of D-aminolevulinate dehydratase in mice.

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