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PURIFICATION AND KINETIC PROPERTIES OF *MUS BOODUGA* (GRAY) HEPATIC ARGINASE

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Hepatic L-arginase from the *Mus booduga* (Gray) was purified and its kinetic characteristics were investigated. The enzyme was not adsorbed on DEAE-cellulose, but was retained on CM-cellulose column at pH 7.2. The Michaelis–Menten constant was 8.3 mM for L-arginine and was independent of pH in the range of 7.5–10.5. L-arginine concentrations as high as 0.4 M did not exert substrate inhibition in the pH range 7.4–10.0. Manganese was required at a concentration of 0.05 M for full activation of the enyme. L-ornithine and L-lysine inhibited the enzyme competitively with inhibitory constants of 1.9 mM and 3.7 mM respectively. Several properties of the L-arginase from *Mus booduga* clearly identify it as an enzyme similar to ureotelic basic arginases from mammalian liver.

Keywords: Arginase; Kinetics; *Mus booduga*; Enzyme inhibition; Purification

INTRODUCTION

The enzyme arginase (L-arginine ureohydrolase, EC 3.5.3.1) catalyzes the hydrolytic conversion of L-(+)-arginine to ornithine and urea. This reaction in the Krebs–Hanseleit cycle, is a mechanism by which the nitrogen derived from the amino acid and ammonia is converted to urea in the mammalian liver thereby regulating ammonia levels.[1,2] The arginase has been prepared in states of high purity from the livers of various ureotelic and uricotelic animals.[3–5] Remarkable differences are known to exist

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between ureotelic and uricotelic arginases with respect to their molecular weights, K_m values, inhibition by excess substrate and antigenicity.[6,7] The tetrameric structure of the rat hepatic and kidney arginases has been clearly documented.[7] Recently Porembska and Zamecka[8] characterized five immunologically different forms of arginases in rat tissues through the double-diffusion test and immunoelectrophoresis. New symbols for these arginases were proposed (beginning with most anionic forms): A_1 (kidney), A_2 (liver), A_3 (salivary glands), A_4 (kidney) and A_5 (liver). Arginases A_1 from kidney and A_5 from liver were parental forms built of one type of subunit. Subunits of the A_1 form exhibited non-identical cross reaction with subunits of the A_5 form. Arginase A_2, A_3, A_4 were hybrids composed of both kinds of subunits. Classification of arginases into two groups, 'ureotelic' and 'uricotelic'[9] does not seem justified because of the lack of known correlation between the mode of nitrotelism.[10] Others have reported differences in the characteristics of hepatic arginases of different mammals.[11] Despite extensive work made on the enzyme with crude or purified or crystalline preparations, it still remains ill-defined in terms of its basic molecular properties in different species. The present investigation deals with the purification and some basic kinetic properties of the Indian field mouse, *Mus booduga* (Gray) hepatic arginase.

MATERIALS AND METHODS

Enzyme Assays

The hepatic arginase (EC 3.5.3.1, L-arginine ureohydrolase) activity was determined by measurement of urea produced, by using diacetyl monoxime reaction as followed by Paik *et al.*[12] A fraction of the enzyme extract (crude/purified aqueous preparation) was heated with an equal volume of 0.1 M $MnSO_4$, pH 7.0 (a final concentration of 0.05 M $MnSO_4$) at 55°C for 10 min in order to activate the enzyme.[13] A purified preparation containing 1.7 units of arginase (unless otherwise stated) or 200 µl of 10% homogenate supernatant was incubated at 37°C for 10 min with 0.15 M L-arginine (unless otherwise stated) preadjusted to pH 9.5 with 1 M HCl; glycine–NaOH buffer (0.1 M, pH 9.5) and $MnSO_4$ (0.01 M). The reaction was stopped by the addition of 2.5 ml of 10% $HClO_4$. To 0.5 ml of the clear supernatant obtained after the centrifugation of the above mixture, 5 ml of H_2SO_4:H_3PO_4 (1 : 3) mixture was added followed by 0.5 ml of 0.1 M $FeCl_3$ and 1 ml of 0.75% diacetyl monoxime in ethanol. The contents were thoroughly mixed in vortex-200 cyclomixer and boiled in a hot

water-bath at 90°C for 15 min. After cooling in an ice-bath for 5 min the samples were read at 480 nm against reagent blank. The enzyme unit was defined as the amount of enzyme that produced 1 μmole of urea per min at 37°C. Specific activity was expressed in enzyme units per mg protein. The protein content of the enzyme source was estimated by the method of Lowry *et al.*[14] using crystalline bovine serum albumin as standard. Modifications of the basic assay or activation procedures are given wherever applicable.

Purification of *Mus Booduga* Hepatic Arginase

The scheme outlined in Table I representing several steps as described by Schimke[15,16] for the purification of rat liver arginase, was adopted for the purification of *Mus booduga* hepatic arginase with some modifications. Unless otherwise stated the following purification steps were carried out at 5°C.

1. *Extraction*: The sample of liver weighing 6.5 g was homogenized in 9 vol. of solution containing 0.2 M KCl, 0.01 M $MnSO_4$ and 0.01 M tris-Cl pH 7.0. The homogenate was centrifuged at 20 000 × *g* for 20 min at 4°C and the supernatant was collected. This residue was washed twice with the homogenizing medium and the supernatants were pooled.

2. *Acetone powder*: The pooled extract was brought to 0°C and 4 vol. acetone was added slowly with constant stirring. The addition of the acetone was carried out in a flat bottomed flask jacketed with freezing mixture at −10°C. The precipitate collected by centrifuging at 15 000 × *g* for 10 min at −10°C was air-dried at room temperature for 30 min or longer and stored or subjected to immediate extraction.

3. *Dialysis and DEAE-cellulose chromatography*: 1 g of liver acetone powder was extracted with three 15 ml portions of 0.005 M tris-Cl, pH 7.2, containing 0.01 M $MnSO_4$. The sediment was removed by centrifugation.

The dialysis tube was pre-processed as follows. The desired length of the tube was allowed to simmer (70°C) in 50% ethanol for 2 h in 2 changes. Then the tube was immersed in 10 mM sodium bicarbonate for 2 h and in 1 mM EDTA for 1 h. The tube was then immersed in double distilled water for 1 h in 2 changes and finally transferred into a separate container of double distilled water and kept at 4°C.

The acetone powder extract was centrifuged, and the clear supernatant was dialyzed against 5 mM tris-Cl pH 7.2 for 3–4 h.

The DEAE-cellulose treatment was as described by Schimke,[15] except that the acetone powder extract was dialyzed (as described above) prior to

TABLE I Purification scheme for the *Mus booduga* hepatic arginase

Step	*Protein*			*Enzyme*			*Recovery* (%)	*Apparent purification factor*
	Volume of fraction (ml)	*Concentration* (mg/ml)	*Total amount* (mg)	*Concentration* (units*/ml)	*Specific activity* (units*/mg protein)	*Total amount* (units)*		
Crude homogenate	63.0	25.00	1575	97	3.9	6 111	100	1.0
Homogenate supernatant	76.0	8.70	661.2	83	9.5	6 308	103	2.5
Acetone powder extract	41.0	2.4	98.4	134	56	5 484	90	14
DEAE cellulose	33.5	0.85	28.5	128.7	150.0	4 278	70	39
CM-cellulose	58.0	0.14	8.1	548	391	3 178	52	101
Ammonium sulphate	3.2	0.44	1.4	458	1048	1 467	24	270
Acetone	1.6	0.513	0.821	641	1249	1 026	17	322

* μmole of urea × min^{-1} at 37°C.

the treatment with DEAE-cellulose. The equilibration of DEAE-cellulose column was brought about with 5 mM tris-Cl, pH 7.2.

4. *Activation*: The DEAE-cellulose column effluent was heated at 55°C for 20 min after adding $MnSO_4$ to a final concentration of 0.01 M to activate the enzyme, and the protein that was precipitated was removed by centrifugation.

5. *CM-cellulose chromatography*: The CM-cellulose was prepared from Whatmann cellulose microcyrstalline, as described by Peterson and Sober.[17] The ion exchanger was pre-processed as described by Schimke[15] and equilibrated with 0.005 M tris-acetate pH 6.2. The DEAE-cellulose column effluent was placed on a CM-cellulose column. To elute the adsorbed protein, a stepwise elution was performed using different concentrations of NaCl solutions (0.05–0.3 M in 0.005 M tris-acetate, pH 6.2). To the fractions containing arginase 0.1 M $MnSO_4$ adjusted to pH 7.0 was added to get a final concentration of 0.01 M $MnSO_4$.

6. $(NH_4)_2SO_4$ *fractionation*: Arginase was precipitated from the active fractions with ammonium sulphate with concentration between 2.0 M and 2.5 M. The precipitate was taken up in 5% of the original extract volume in 0.005 M tris-Cl pH 7.0 containing 0.01 M $MnSO_4$.

7. *Acetone treatment-II*: Arginase was precipitated with an equal volume of cold acetone. The precipitate was dissolved in 2.5% of the original extract volume in 0.005 M tris-Cl, pH 7.0 containing 0.01 M $MnSO_4$. Any sediment present was removed by centrifugation.

RESULTS AND DISCUSSION

Enzyme Purification

The steps for the purification of *Mus booduga* liver arginase are summarized in Table I. The specific activity in the crude homogenate was 3.9 units mg protein^{-1}. A gradual increase in specific activity in subsequent steps of purification is shown in Table I. The purification through this series of procedure yielded enzyme with a specific activity of 1249 units mg protein^{-1} after activation in 50 mM $MnSO_4$ pH 7.0 at 55°C for 5 min.

Chromatographic Behaviour of *Mus Booduga* Hepatic Arginase

Mus booduga hepatic arginase was not adsorbed on a DEAE-cellulose column equilibrated with 0.005 M tris-Cl, pH 7.2, but was retained on a CM-cellulose column. The adsorbed enzyme was eluted stepwise with

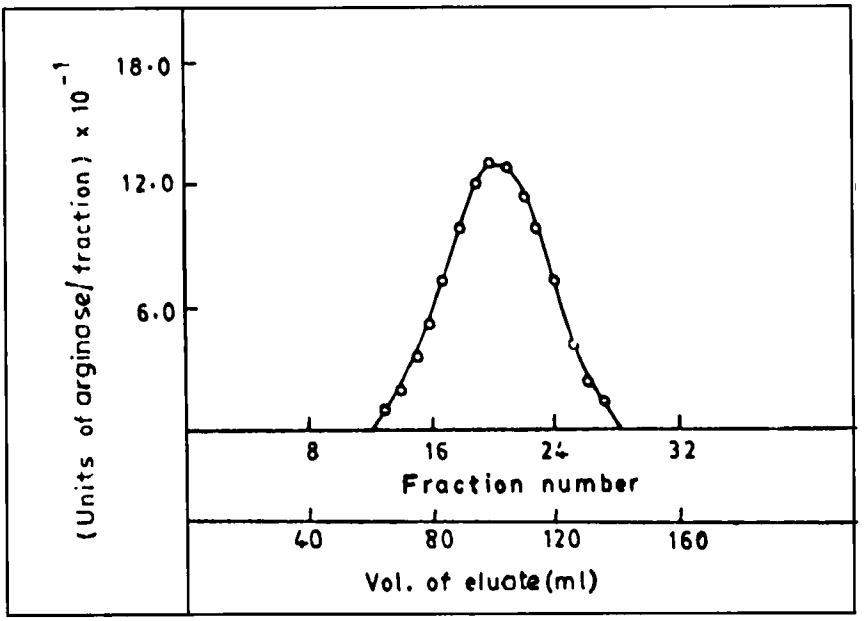

FIGURE 1 Elution profile of *Mus booduga* hepatic arginase from CM-cellulose column, equilibrated with 0.05 M tris-acetate pH 6.2. Pooled fractions from 12 to 28 yielded a specific activity of about 391 units mg protein^{-1}.

varying concentrations (0.05–0.3 M) of NaCl. Adsorption behaviour of different types of arginases was studied by Hirsh-Kolb *et al.*[18] who suggested that slightly acidic or neutral arginases were not adsorbed on a CM-cellulose column and were eluted from the column with void volume, whereas the basic arginases were tightly bound to the CM-cellulose column and the active protein was eluted with either L-arginine or with dilute salt solutions. The chromatographic behaviour of *Mus booduga* hepatic arginase suggests that it may be a basic type of arginase. The elution pattern of the enzyme from the CM-cellulose column, is shown in Figure 1. Appearance of a single peak suggests that the *Mus booduga* hepatic arginase is a single entity.

Substrate Kinetics

Arginase was assayed over a range of 10–100 mM L-arginine (preadjusted to pH 9.5 with 1 M HCl) at 37°C. As the concentration of L-arginine in the standard assay medium was varied, glycine (preadjusted to pH 9.5 with

0.2 M NaOH) was added to the reaction mixture in such a way as to keep the total concentration of amino acids (arginine + glycine) constant at 250 mM. The results showed that the metabolism of L-arginine was dependent on its concentration up to 50 mM. At L-arginine was carried out because inhibition by high substrate concentrations was considered a property of arginase from ureotelic animals.[9] This property is however controversial since other workers could not demonstrate the substrate inhibition of rat hepatic arginase.[19] *Mus booduga* hepatic arginase did not exhibit inhibition at concentrations of L-arginine upto 0.4 M in the range of pH 7.4 to 10.0 and the ratios of activities at high (0.4 M) and low (0.1 M) concentrations of L-arginine were constant (1.12) (Figure 2). The Michaelis–Menten constant (K_m) was determined by employing the method of least squares as the best fit.[20] For *Mus booduga* hepatic arginase a K_m value of 8.3 mM was obtained at pH 9.5. This K_m value lies in the same range proposed for various vertebrate species and is quite similar to the K_m value (6.3 mM) proposed by Kayser and Strecker[19] for rat enzyme. For mouse hepatic arginase a K_m of 6.0 mM was reported by Hirsh-Kolb *et al.*[18] All these values (6–20 mM) are of the same order of magnitude as proposed by Mora *et al.*[9] for a number of ureotelic species.

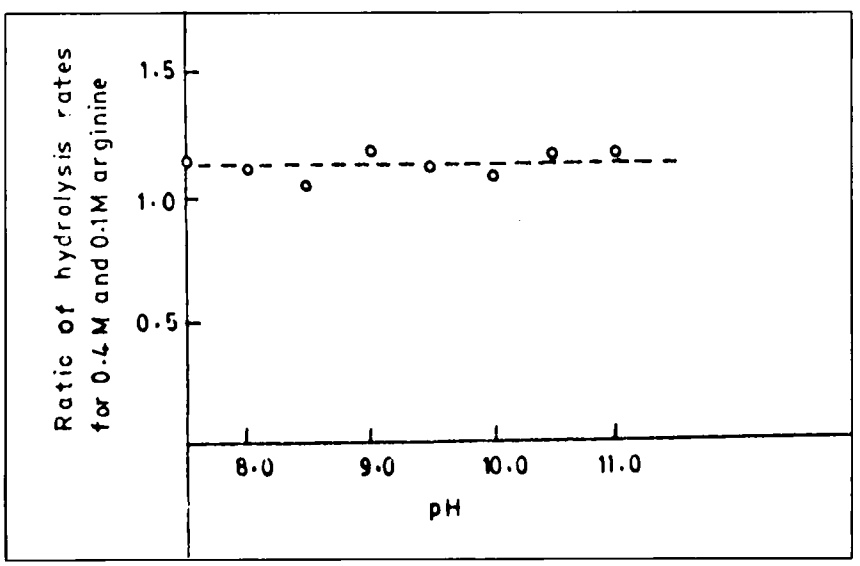

FIGURE 2 Relative rates of hydrolysis by *Mus booduga* hepatic arginase at L-arginine concentrations of 0.4 M and 0.1 M.

Inhibitors

A number of substrate analogues, amino acids, products of ornithine metabolism and other compounds were reported to inhibit liver arginase.[21,22] As shown in Tables II and III both L-ornithine and L-lysine inhibited the *Mus booduga* hepatic arginase. In both cases the inhibition was a purely competitive type which was characterized by an increased K_m and an unchanged V_{max}. Under defined conditions and at an equimolar ratio (1:1) of substrate and inhibitor (L-ornithine or L-lysine), the inhibition of the activity was 72.4% and 65.3% with L-ornithine and L-lysine respectively. With an increase in substrate concentration, the inhibitory effect decreased, and at a molecular ratio (substrate : inhibitor) of 32 : 1 the inhibitory effect of L-lysine on arginase activity was completely removed, whereas a relatively high concentration of substrate (molecular ratio of 64 : 1) was required to overcome the inhibitory effect of L-ornithine completely.

TABLE II Inhibition of *Mus booduga* hepatic arginase by L-lysine

Lysine concentration (M)	*Molecular ratio (substrate : lysine)*	*Arginase activity* (μmole urea/min)	*Inhibition* (%)
0		1.7 ± 0.10	0
4.7×10^{-3}	64 : 1	1.7 ± 0.16	0
9.4×10^{-3}	32 : 1	1.7 ± 0.14	0
18.8×10^{-3}	16 : 1	$1.5 \pm 0.09^{***}$	11.8
37.5×10^{-3}	8 : 1	$1.22 \pm 0.09^{*}$	28.2
75×10^{-3}	4 : 1	$0.96 \pm 0.06^{*}$	43.5
150×10^{-3}	2 : 1	$0.75 \pm 0.04^{*}$	55.9
300×10^{-3}	1 : 1	$0.59 \pm 0.04^{*}$	65.3

The L-arginine concentration in the basic assay system was increased to 0.3 M. Activity values are means, ± S.D. of six observations. *t*-test: $^{*}P < 0.001$; *** insignificant difference from control.

TABLE III Inhibition of *Mus booduga* hepatic arginase by L-ornithine

Ornithine concentration (M)	*Molecular ratio (substrate : ornithine)*	*Arginase activity* (μmole urea/min)	*Inhibition* (%)
0		1.7 ± 0.13	0
4.7×10^{-3}	64 : 1	1.7 ± 0.13	0
9.4×10^{-3}	32 : 1	$1.6 \pm 0.10^{***}$	5.9
18.75×10^{-3}	16 : 1	$1.42 \pm 0.06^{*}$	16.5
37.5×10^{-3}	8 : 1	$1.07 \pm 0.03^{*}$	37.1
75×10^{-3}	4 : 1	$0.91 \pm 0.03^{*}$	46.5
150×10^{-3}	2 : 1	0.69 ± 0.05	59.4
300×10^{-3}	1 : 1	$0.47 \pm 0.04^{*}$	72.4

The L-arginine concentration in the basic assay system was increased to 0.3 M. Activity values are means, ± S.D. of six observations. *t*-test: $^{*}P < 0.001$; *** insignificant difference from control.

The kinetics of inhibition of the hepatic arginase by ornithine and lysine are given in Figures 3 and 4 in the form of Lineweaver-Burk double reciprocal plots. The *Mus booduga* hepatic arginase showed a greater affinity for ornithine than for lysine. The inhibitory constants (K_i) estimated from the data given in Figures 3 and 4 were 1.9 mM and 3.7 mM for ornithine and lysine respectively. Campbell[23] reported the K_i for ornithine to be 1.34 mM and for lysine, 2.37 mM ($K_m = 5.4$ mM) for the rat liver enzyme which was competitively inhibited by both the amino acids. A higher value of K_i for L-lysine than for L-ornithine was also demonstrated with beef liver enzyme.[24] The results obtained in the present study are consistent with the concept that the *Mus booduga* liver arginase shows many similarities with the rat enzyme in kinetic characteristics and amino acid inhibition patterns.

Activation

In order to obtain the maximum activity of hepatic arginase in a purified or partially purified preparation, pre-incubation with Mn^{2+} ions is needed,[15] though this is not necessary in crude preparations if the Mn^{2+} ions

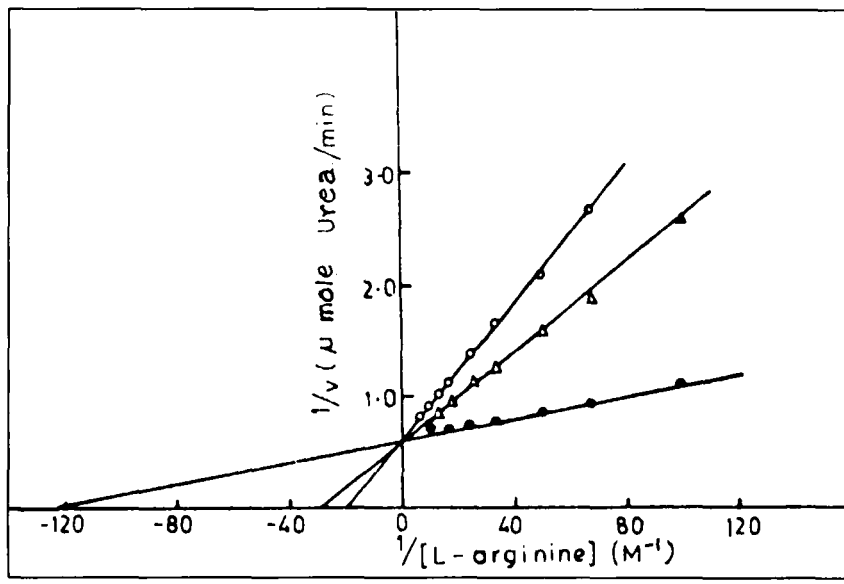

FIGURE 3 Double reciprocal plots showing the competitive inhibition of *Mus booduga* hepatic arginase (1.7 units mg protein^{-1}) by L-ornithine; (●) control; (△) 5 mM; (○) 10 mM.

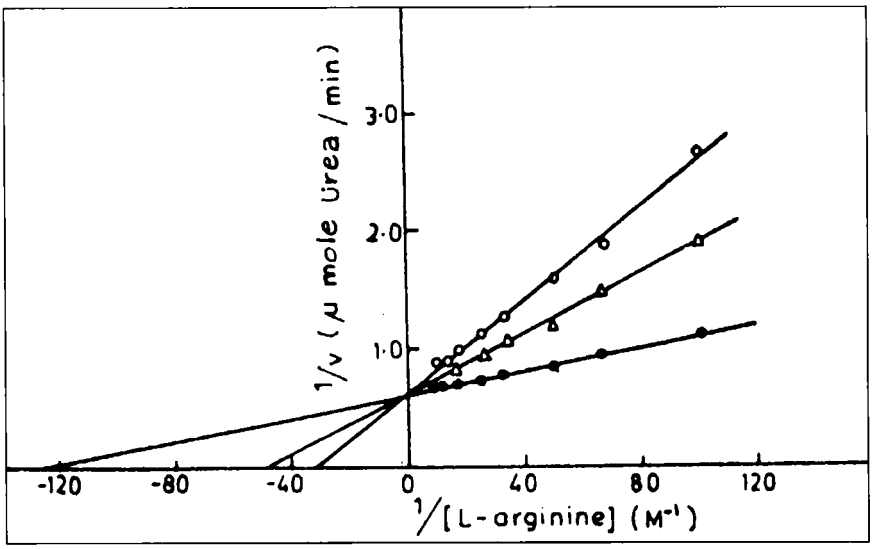

FIGURE 4 Double reciprocal plots showing the competitive inhibition of *Mus booduga* hepatic arginase (1.7 units mg protein^{-1}) by L-lysine; (●) control; (△) 5 mM; (○) 10 mM.

are included in the homogenizing medium.[3] The activation process involves the reversible transformation of an inactive protein-proarginase to arginase according to the equation:

$$\text{Proarginase} + M^{2+} \longleftrightarrow \text{Arginase} - M^{2+}.$$

The metal, M, is generally considered[4,25] to be Mn^{2+}, Co^{2+} or Ni^{2+}. The dependence of activation of *Mus booduga* liver arginase on manganese ion concentration is shown in Figure 5. For this study, a clear supernatant from the liver homogenate made in tris-Cl buffer not containing manganese ions was employed. Varying concentrations of $MnSO_4$ were added to the enzyme extract and the activation was carried out at 55°C for 10 min. The results show that the optimal concentration of Mn^{2+} under these conditions is 50 mM.

When the dilute purified *Mus booduga* hepatic arginase samples containing 0.005 M Mn^{2+} were dialyzed against 0.005 M tris-Cl buffer (pH 7.0) with daily changes of the dialysis bath, the catalytic activity slowly decreased (Figure 6). Within two days of dialysis, 45% of the activity was lost. No further loss of activity was observed if the dialysis process was continued up to 4 days. When reactivated with Mn^{2+}, 85–95% of the

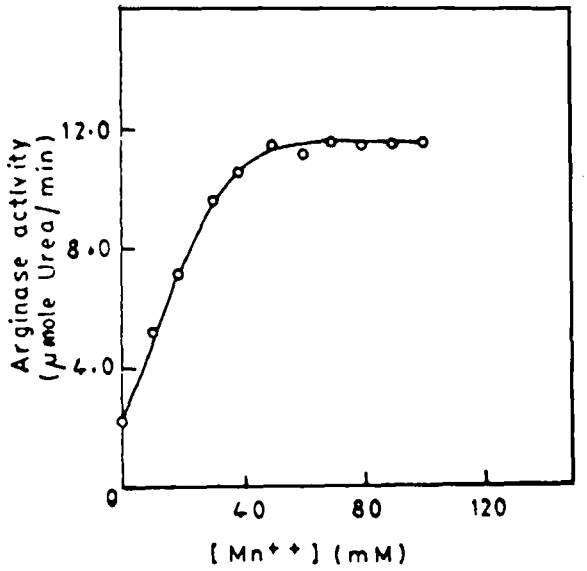

FIGURE 5 Dependence of the activation of *Mus booduga* hepatic arginase on manganese ion concentrations. A clear supernatant of the homogenate prepared in 10 mM tris-Cl pH 7.0 containing 0.2 M KCl was used. The activation was for 10 min at 55°C.

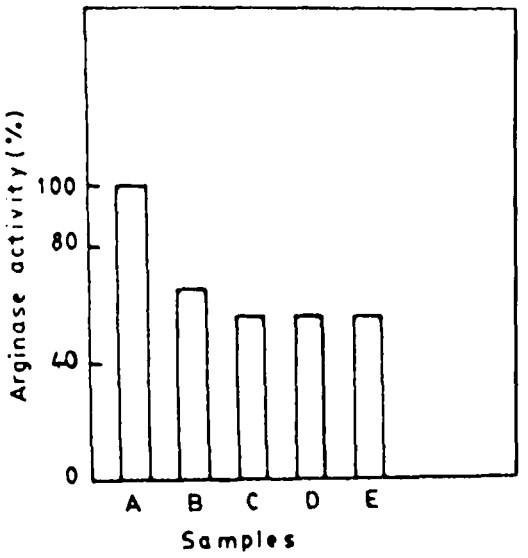

FIGURE 6 Loss of *Mus booduga* hepatic arginase (dilute purified preparation) activity by prolonged dialysis against 0.005 M tris-Cl buffer pH 7.0, at 4°C for zero (A); one (B); two (C); three (D) and four (E) days. The activity of fully Mn^{2+} activated original sample was taken as 100% enzyme activity.

original activity could be regained in all the dialyzed samples. These observations suggest that the activation of *Mus booduga* hepatic arginase is a reversible reaction and the loss of activity during dialysis may be due to the removal of Mn^{2+} from the holoenzyme.[26] Hirsch-Kolb *et al.*[18] reported that the catalytic activity of basic arginases (rat, mouse and dog) is reduced to about 40–50% of the original (maximal) value after 2 days of dialysis, whereas neutral and slightly acidic arginases (beef, monkeys I and II and rabbit) lost more of their catalytic activity (65–85%) under the same conditions. Magnetic resonance studies of rat liver arginase revealed that the catalytic activity was directly correlated to Mn^{2+} binding. The loss of Mn^{2+} obviously renders the enzyme more unstable, which results in a higher denaturation of the enzyme on prolonged dialysis. The results of the present study clearly reveal that *Mus booduga* hepatic arginase binds Mn^{2+} more tightly than the neutral or slightly acidic arginases and hence 55% of the activity was retained after 14 days of continuous dialysis.

Enzyme Stability

The results of the enzyme stability studies suggest that crude preparation of *Mus booduga* hepatic arginase retains its full activity at 55°C for 25 min in the presence of 0.05 M Mn^{2+}. Longer periods of incubation at this temperature resulted in a progressive decrease in the activity. In contrast the same preparation under similar conditions was stable only for 5–10 min in the absence of Mn^{2+}. The purified enzyme was stable for only 10 min at 55°C in the presence of 50 mM Mn^{2+}. If the purified preparation which was dialyzed for 4 days against 0.005 M tris-Cl pH 7.0 was used, a loss of activity was noticed within 5 min (Figure 7). The enzyme exhibited a temperature-dependent binding of Mn^{2+} for the complete activation *in vitro* (Table IV). Supplementation of Mn^{2+} to the extraction medium resulted in 67% of the activity in crude preparations and on heat treatment full activation was attained. The purified enzyme preparation which had lost part of the Mn^{2+} during dialysis was quite unstable to heat and only 18% activity was retained after 5 min of heat treatment, when compared to a similar preparation which was fully active (100%) (Figure 5). The activation energy (ΔE) values of *Mus booduga* hepatic arginase calculated from the Arrhenius plots ranged from 16 731 to 17 082 Cal/mole over 10–60°C temperature range.[27] Hirsch-Kolb *et al.*[18] formed the opinion that the basic arginases found in mammalian species were more stable than slightly acidic or neutral arginases to certain conditions such as dialysis, column chromatography and heat treatment.

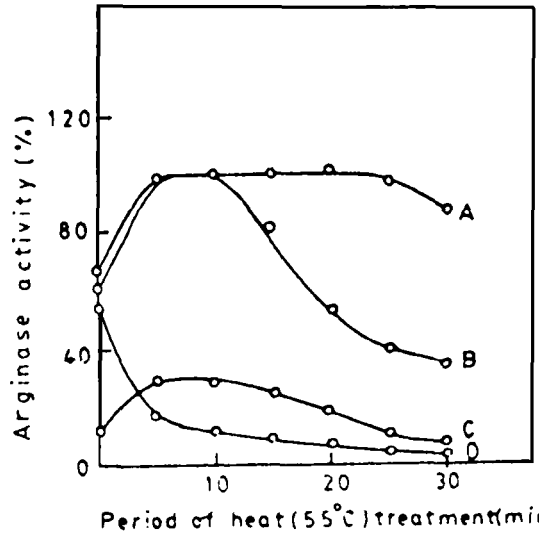

FIGURE 7 Heat stability of *Mus booduga* hepatic arginase. 1 ml of 5% homogenate supernatant (40 units mg protein^{-1} of arginase) was pre-incubated at 55°C for increasing periods in the presence (A) and absence (C) of 0.05 M $MnSO_4$ at pH 9.5. 1 ml of dilute purified preparation (20 units mg protein^{-1} of arginase), previously dialyzed against 0.005 M tris-Cl buffer pH 7.0 for four days was heated at 55°C in the presence (B) and absence (D) of 0.05 M $MnSO_4$ for different time periods, at pH 9.5.

TABLE IV Effect of Mn^{2+} on the heat stability of *Mus booduga* hepatic arginase, crude and purified preparations

Treatment		*Arginase activity* (%)	
Mn^{2+}	*Heat at* 55°C	*Crude preparation*[a]	*Purified preparation*[b]
−	−	12	55[a]
−	+	30	18[b]
+	−	67[a]	62
+	+	100[b]	100[c]

[a] Mn^{2+} was added to the extraction medium prior to the homogenization. [b] Dilute purified sample dialyzed against 0.005 M tris-Cl pH 7.0 for four days was used. [c] The heat treatment was for 5 min at 55°C.

Dependence of Substrate Kinetics on pH

The effect of pH on enzyme activity was determined using an arginine–glycine mixture adjusted to required pH. The activity was increased from pH 7.5 to its maximum at 9.5. Between pH 9.0 and 10.0 the enzyme was more active with a rapid loss of activity on either side of this pH range (Figure 8).

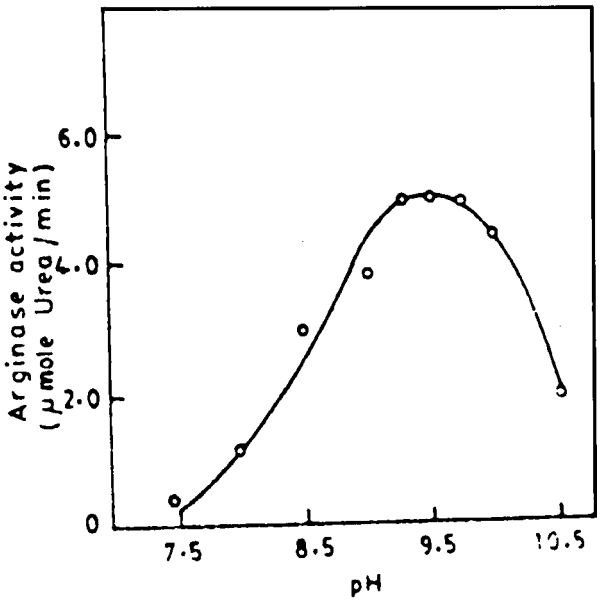

FIGURE 8 Effect of pH on *Mus booduga* hepatic arginase. Dilute purified preparation containing 5.1 units mg protein^{-1} (pH 9.5) of activity was used for the assay. The assay conditions are given under 'experimental' except that the pH of the arginine–glycine mixture was adjusted as required.

Effect of pH on Michaelis–Menten constant was determined by studying kinetics at different pH values. The results (Figure 9) indicated that the Michaelis–Menten constant was unaffected in the pH range 7.5–10.5.

In conclusion, the results of the present study reveal that the *Mus booduga* hepatic arginase shows many similarities to rat liver enzyme in its kinetic properties, probably because an enzyme from different sources functions via same basic catalytic mechanisms as suggested by Dixon and Webb.[28] The chromatographic behaviour of the enzyme confirms that it is a basic type of arginase and is composed of a single entity. The Indian field mouse *Mus booduga* is a ureotelic mammal and the K_m of its hepatic arginase is well fitted in the range of K_m values proposed for various ureotelic animals. There is a close relation between K_m values and antigenic properties. All the arginases present in liver of various ureotelic animals cross-react with the rabbit antibodies prepared against purified rat liver arginase,[9] and they have K_m values of 10–20 mM. The arginases with K_m values of 100–200 mM present in the liver of ureotelic species fail to cross-react with the same antibodies.[9] However, both ureotelic and uricotelic arginases are highly specific for L-arginine.[19]

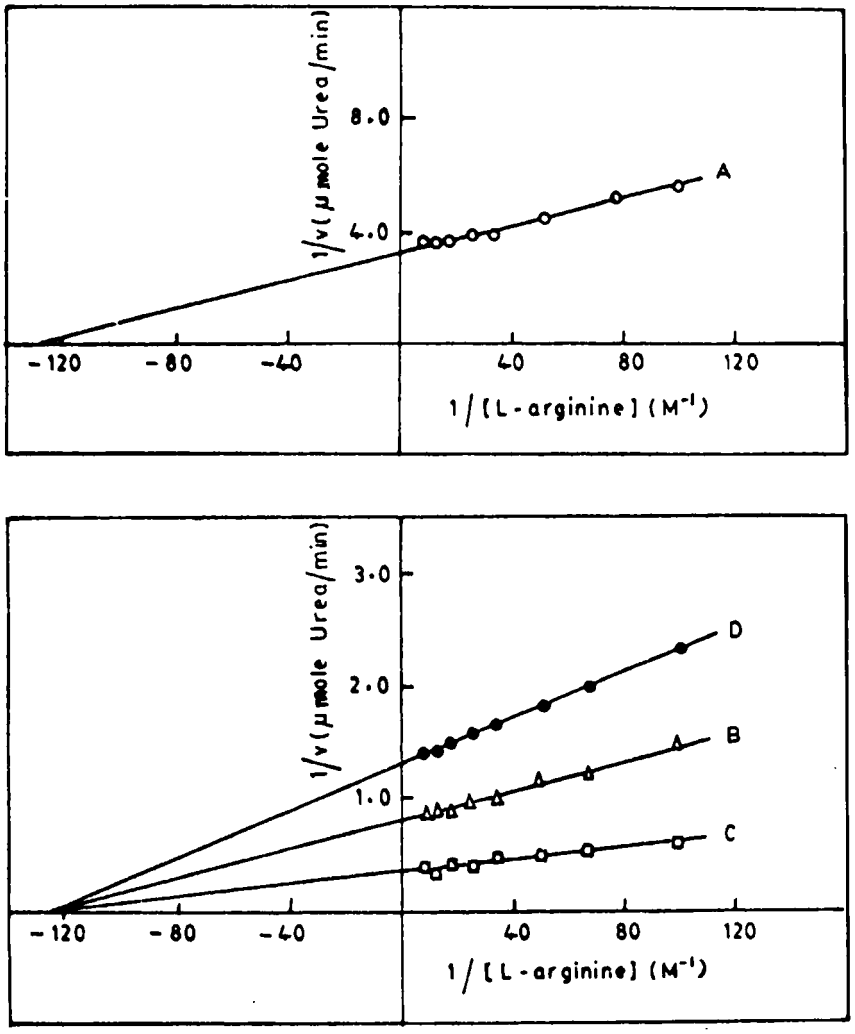

FIGURE 9 Lineweaver-Burk double reciprocal plots showing the effect of pH on the Michaelis–Menten constant of *Mus booduga* hepatic arginase (3 units mg protein^{-1}). (A) pH 9.5; (B) pH 8.5; (C) pH 7.5; (D) pH 10.5.

The competitive inhibition by the product, L-ornithine and its homologue L-lysine (Figures 3 and 4) resemble both qualitatively and quantitatively that of liver enzyme.[29,30] Besides ornithine and lysine, a few other amino acids like valine, leucine, isoleucine and proline inhibit mammalian arginase.[19] Methionine and phenylalanine inhibited bovine liver arginase.[31] Muszynska *et al.*[32] and Mora *et al.*[9] consider that ureotelic and uricotelic

arginases can be distinguished from each other by studying substrate inhibition and the type of inhibition by the product (L-ornithine). According to these workers ornithine is a competitive inhibitor of uricotelic arginase, but a non-competitive inhibitor of ureotelic arginase. This type of distinction is however untenable because hepatic arginase from both ureotelic[30] and uricotelic[21] species were inhibited completely by ornithine.

Since the amino acids act as physiological modifiers by modulating the enzyme, the concentration of free amino acids *in vivo* has considerable significance in metabolic regulation. It can be further suggested that the variability of K_m values between experiments, noted for arginase from other animals,[3] may be due to the differences in the levels of amino acids and other metabolites in the liver extracts of different individuals.

Substrate inhibition of arginase which was considered to be a characteristic of ureotelic arginase[9] could not be demonstrated in rat liver arginase.[19] The hepatic arginase from *Mus booduga* which is ureotelic was not inhibited by concentrations as high as 400 mM arginine. Hence it is suggested that the inhibition of hepatic arginase may not be a characteristic of all ureotelic species.

The enzyme substrate affinities were independent of pH in a pH range 7.5–10.5 and the maximal activity was exhibited at pH 9.5. The activation energy obtained for *Mus booduga* hepatic arginase is 16.9 kcal/mole. Campbell[23] reported a value of 11.6 kcal/mole for rat liver enzyme and values of 9.5 and 10 kcal/mole were reported for human erythrocytes and liver enzymes by Cabello *et al.*[11] The significance of differences in the activation energy of arginases in different species is not well understood because no general pattern is present in the results obtained for enzymes from different sources. According to Dixon and Webb[28] enzymes from different sources generally show different activation energies.

The requirement for Mn^{2+} as a metabolic cofactor appears to be common for several arginases reported, although Co^{2+}, Fe^{2+}, Ni^{2+} may also act as activators[21,33] reported that four Mn^{2+} ions were bound per enzyme molecule of rat liver and the binding was not identical; two ions were loosely bound and their removal caused about 50% loss in activity. The remaining two ions were very tightly bound and their removal caused irreversible inactivation of the enzyme. Luisa *et al.*[5] through their nuclear magnetic resonance studies showed that in calf hepatic arginase 2 Mn^{2+} ions are weakly bound; one Mn^{2+} is strongly bound and the fourth Mn^{2+} is so tightly bound that it is very difficult to remove under the experimental conditions used. However human liver arginase[34] and rabbit liver arginase[35] apparently bind all four Mn^{2+} with equal affinity, and the

removal results in the denaturation of the enzyme. The purified preparation of *Mus booduga* hepatic arginase showed complete activation at 0.05 M Mn^{2+} when heated at 55°C for 5 min. Dialysis of the activated enzyme against the manganese-free medium resulted in a 45% loss in activity which is a characteristic feature of basic arginases (e.g., rat, mouse and dog). The neutral or slightly acidic arginases (e.g., beef, monkeys I and II, rabbit and human), however lose 70–90% activity during dialysis under similar conditions[18] because they lose Mn^{2+} to a greater extent, thus rendering the enzyme more unstable on prolonged dialysis.

Despite some minor variations, several properties including chromatographic behaviour and kinetic characteristics clearly identify the hepatic arginase from *Mus booduga* as an enzyme similar to ureotelic basic arginases from mammalian (especially rat) liver. However various other molecular characteristics of *Mus booduga* arginase including the electrophoretic behaviour, amino acid composition, molecular weight, immune responses, subunit properties etc., are to be investigated for clear understanding of arginase of this species.

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