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Received March 15th, 1968

Biochim. Biophys. Acta, 159 (1968) 554–557

BBA 63328

A low molecular weight arginase in the earthworm

Mammalian liver arginase (L-arginine ureohydrolase, EC 3.5.3.1) has a molecular weight of about 140 000 (refs. 1, 2) whereas the molecular weights of the arginases present in chicken (*Gallus domesticus*) and lizard (*Ctenosaura pectinata*) liver and *Neurospora crassa* are around 280 000 (refs. 3, 4). In this report, we show that the arginase in the earthworm (*Lumbricus terrestris*) gut is unique in being a much smaller protein than any of the previously studied arginases. The metabolic role and certain properties of this enzyme have been described[5]. The molecular weight of earthworm arginase is estimated to be around 27 000. Because it is in the size range of several protein subunits[6], the earthworm enzyme could conceivably represent the natural occurrence of a "monomer unit" of arginase.

The molecular weight of earthworm gut arginase, present in unfractionated tissue extracts, was estimated from data obtained by density gradient centrifugation and gel filtration[7,8]. For gel filtration studies, gut tissue was homogenized in an equal volume 0.1% (w/v) cetyltrimethylammonium bromide with a Potter–Elvehjem tissue grinder. The homogenate was then diluted with a solution containing 50 mM Tris chloride (pH 7.5), 100 mM KCl and 5 mM $MnCl_2$ to give a final tissue concentration of 20% (w/v). This Tris–KCl–$MnCl_2$ solution was also used as the eluting buffer for the Sephadex columns. The diluted homogenate was centrifuged at 27 000 $\times$ *g* for 20 min at 0°. A portion (3–4 ml) of the supernatant fluid was placed on the column after sucrose (5 mg/ml) had been added to increase its density. For density gradient centrifugation, the gut tissue was homogenized in a solution containing 50 mM Tris chloride (pH 7.5) and 5 mM $MnCl_2$ to give a 20% tissue homogenate. This Tris–$MnCl_2$ solution was also used for preparing the sucrose gradients. The homogenate was centrifuged as above and a portion (0.2 ml) of the supernatant fluid was layered on the gradient. The same procedures were followed with rat liver and snail hepatopancreas, except that 10% tissue homogenates were prepared.

Columns of Sephadex G-100 (Pharmacia, Uppsala) were prepared and calibrated as described by ANDREWS[9]. The size of the column was 2.5 cm $\times$ 50 cm and the temperature was maintained at 0° with a water jacket. Elution was with the Tris–KCl–$MnCl_2$. solution and 3 ml fractions were collected. Gel filtration data were calculated as the parameters (K_D ref. 10) and K_{av} (ref. 11). Plots of either $K_D^{\frac{1}{3}}$ *vs.* Stokes radius or

$(-\log K_{av})^{\frac{1}{2}}$ *vs.* Stokes radius were linear[8]. The Stokes radii of the calibrating proteins (Fig. 1), except apoferritin, were taken from LAURENT AND KILLANDER[11]. The Stokes radius of apoferritin was calculated using the diffusion coefficient reported by ROTHEN[12]. Neither the V_e nor the elution profile symmetry of enzymes of known molecular weight was changed when they were mixed with tissue extracts.

Linear gradients of from 5 to 20% (w/v) sucrose were prepared in the Tris–$MnCl_2$ solution as described by MARTIN AND AMES[7]. The gradient volume was 4.8 ml. The gradients were centrifuged at 38 000 rev./min for 16 h at 4° in an SW 50 rotor with the Spinco Model L-2 centrifuge. Fractions of approx. 0.17 ml each were collected from the gradients for enzyme assay.

Arginase activity was determined by previously described methods[13]. Urea formation was measured for fractions from Sephadex columns and ornithine formation for fractions from sucrose gradients. Enzyme units are μmoles product per mg protein per h at 25°.

The gel filtration behavior of earthworm gut and rat liver arginases is shown in Fig. 1. Mixing experiments showed that the earthworm arginase could be separated from other arginases by gel filtration. For example, when earthworm gut extracts were mixed with extracts of land snail (*Otala lactea*) hepatopancreas, two distinct and

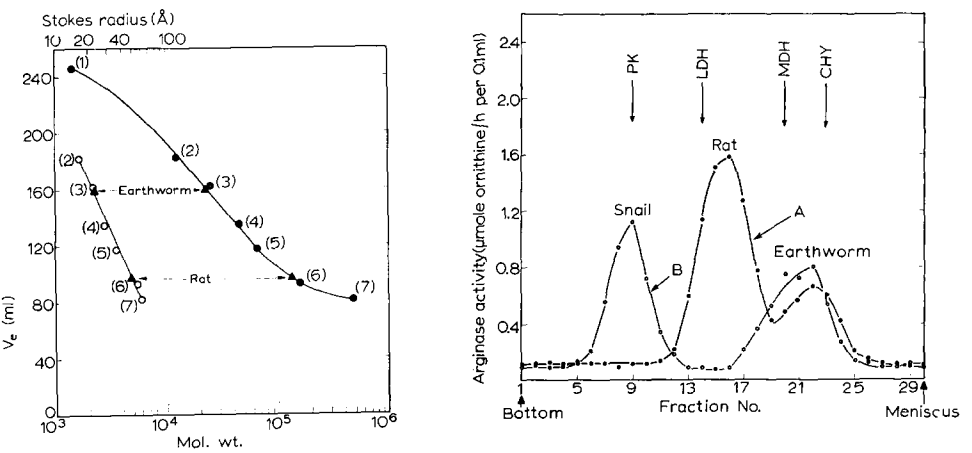

Fig. 1. Gel filtration behavior of earthworm gut and rat liver arginases on Sephadex G-100. The calibrating proteins and their molecular weights are: (1) bacitracin, 1450; (2) horse heart cytochrome *c*, 12 400; (3) beef pancreas chymotrypsinogen A, 25 000; (4) ovalbumin, 45 000; (5) bovine serum albumin, 67 000; (6) human γ-globulin, 160 000; and (7) horse apoferritin, 480 000. ○, Stokes radius *vs.* elution volume (V_e); ●, mol. wt. *vs.* V_e. The void volume (V_0) for the column was 76 ml and was determined with Dextran blue 2000 (Pharmacia).

Fig. 2. Separation of earthworm arginase from rat liver arginase (A) and snail hepatopancreas arginase (B) by sucrose density gradient centrifugation. In gradient A, 155% of the added rat liver arginase activity was recovered and 86% of the earthworm gut arginase activity. In gradient B, 119% of the earthworm arginase activity was recovered and 140% of the snail activity. The vertical arrows indicate the fractions in which the calibrating enzymes appear. The molecular weights used for these were: rabbit muscle pyruvate kinase (PK), 244 000 (refs. 15, 16); beef heart lactate dehydrogenase (LDH), 140 000 (ref. 17); pig heart malate dehydrogenase (MDH), 70 000 (ref. 18); and α-chymotrypsin (CHY), 22 000 (ref. 19). The activities of these enzymes were determined as described in refs. 20, 21, 22 and 23, respectively. By gel filtration on Sephadex G-100, we found molecular weights of 24 500, 66 000 and 120 000 for α-chymotrypsin, malate dehydrogenase and lactate dehydrogenase, respectively.

TABLE I

COMPARISON OF MOLECULAR PARAMETERS OF RAT AND EARTHWORM ARGINASES

Enzyme	*Gel filtration*		*Density gradient centrifugation*		*Calculated**	
	Mol. wt.	*Stokes radius (Å)*	*Mol. wt.*	$s_{20,w}$	*Mol. wt.*	f/f_0
Earthworm gut arginase	25 000	22.2	27 000	2.9	29 500	1.04
Rat liver arginase	145 000	47.3	127 000	6.2	134 000	1.38

* The partial specific volume of beef liver arginase was calculated using the diffusion coefficient reported by GREENBERG, BAGOT AND ROHOLT[1]. This value was assumed for both the rat and earthworm enzymes in calculating their molecular weight and f/f_0 from the Svedberg equation[8].

symmetrical peaks of arginase activity emerged from the column. The V_e of each peak was the same as when the individual extract was passed through the column.

As shown in Fig. 2, earthworm gut arginase could also be separated from other arginases by sucrose density gradient centrifugation.

The recovery of arginase activity from both the columns and gradients was usually greater than 100%. This is presumed to be due to the low-temperature activation of the enzymes by Mn^{2+} present in the buffer solutions[13,14].

A comparison of the molecular parameters estimated here for rat liver and earthworm gut arginases is shown in Table I. GREENBERG, BAGOT AND ROHOLT[1] reported an $s_{20,w}$ of 5.95 for beef liver arginase, corresponding to a molecular weight of 138 000, and an f/f_0 of 1.47. The values estimated here for the rat liver enzyme are close to these values. The value of approx. 27 000 (av. of 3 values in Table I) for the earthworm enzyme is the lowest molecular weight thus far reported for an arginase. This low molecular weight is not typical of invertebrate arginase since the earthworm enzyme could be separated from the snail enzyme by both gel filtration and density gradient centrifugation. Snail arginase has a molecular weight of approx. 244 000 (Fig. 2). It is not absolutely certain that the low molecular weight of the earthworm enzyme is not due to dissociation although there was no indication of a higher molecular weight arginase in this species under several different experimental conditions. Even if dissociation were the contributing factor, it is nevertheless significant that an active arginase unit of this small dimension exists since the subunit structure of arginase is not known.*

Supported by grants from the National Science Foundation (GB-4524) and U.S. Public Health Service (AI 05006). J.W.C. is a U.S. Public Health Service Career Development Awardee (5-K3-GM-6780). S.R.R.R. is the recipient of a Fulbright travel grant from the U.S. Educational Foundation in India.

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* *Note added in proof*: HIRSCH-KOLB AND GREENBERG[24] report the subunit weight of rat liver arginase to be 30 800 (Received June 4th, 1968).

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Received March 29th, 1968

Biochim. Biophys. Acta, 159 (1968) 557–560

BBA 63330

Serine dehydratase activity in livers of phlorrhizin-treated rats and the hepatic serine *plus* threonine concentration

The activities of serine and threonine dehydratase are known to increase in rat liver in situations involving intensive protein catabolism, such as starvation, alloxan-diabetes or a high-protein diet, and furthermore the same protein reacts with both substrates[1–3].

Since the administration of phlorrhizin to starved rats results in a rapid increase (within 2.5 h) in the hepatic concentration of serine *plus* threonine, whereas the concentrations of certain other glucogenic amino acids decrease[4], it seemed of interest to examine the activity of serine dehydratase (L-serine hydro-lyase (deaminating), EC 4.2.1.13) in this situation where the amino acids arising from increased protein catabolism in extra-hepatic tissues are expected to contribute to the high rate of hepatic gluconeogenesis.

Male rats of the Wistar strain, weighing 120–170 g, were fed a diet of commercial rat cubes containing about 15% protein, 3% fat and the remainder consisting mainly of carbohydrate. Adrenalectomized rats received 1% NaCl in the drinking water and were used 4–5 days after the adrenalectomy. Phlorrhizin (75 mg) was administered