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Microheterogeneity of molecular forms of arginase in mammalian tissues

Gita Venkatakrishnan[1], V Shankar[2] and S R R Reddy[1*]

[1]Department of Zoology, University of Pune, Pune 411 007, India
[2]Division of Biochemical Sciences, National Chemical Laboratory, Pune 411 008, India

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Two isoforms of arginase, A_1 and A_2, were found in rat liver, submaxillary gland and kidney as well as beef kidney. In beef liver, however, A_2 was the only detectable form. Two additional forms, A_3 and A_4, found only in rat kidney were probably artifactitious. A_1 and A_2 exhibited chromatographic and immunological microheterogeneity. While A_1 in rat liver and submaxillary gland was excluded by DEAE-cellulose (*p*H 8.3) and retained on CM-cellulose (*p*H 7.5), that (A'_1) in beef and rat kidneys was excluded by both ion-exchangers. A_2 in all tissues was retained on DEAE-cellulose, but not on CM-cellulose. Both A_1 and A_2 in rat liver and beef kidney, A_1 from rat submaxillary gland and A_2 from beef liver were precipitated by antibodies to rat and beef liver arginases. None of the forms in rat kidney (A_1, A_2, A_3 and A_4) showed any cross-reactivity to either antibody. Rat submaxillary gland A_2 was precipitated by anti-rat liver arginase, but activated by anti-beef liver arginase. While the major molecular forms were A_1 in rat liver and submaxillary gland and A_2 in beef liver and rat kidney, the two forms occurred in equal proportions in beef kidney. It appears that different isoforms might function as components of the urea cycle in the liver of different mammals and of the arginine catabolic pathway in different extrahepatic tissues.

Keywords: Arginase isoforms; mammalian tissues, chromatographic behaviour; immunological characterization, kinetic and physical properties, activation by antibodies, microheterogeneity.

Arginase (L-arginine amidinohydrolase, EC 3.5.3.1), which hydrolyzes L-arginine to ornithine and urea, is widely distributed in mammalian tissues, both hepatic and extrahepatic[1]. Although this enzyme is conventionally looked upon as a component of the urea cycle in the liver, its presence in non-hepatic tissues that lack a functional urea cycle suggests its involvement in the production of ornithine as a precursor for the synthesis of proline, glutamate and polyamines[2]. It is now recognized that type I and type II arginases, coded by separate genes, serve in the ureogenic and ornithinogenic functions respectively in the liver and extrahepatic tissues like kidney[3, 4].

Earlier reports on arginase isoenzymes, though extensive[4], suffer from several inconsistencies with respect to their number and identity in different mammalian tissues. For e.g., based on DEAE-cellulose chromatography (*p*H 8.3), Porembska and co-workers[5, 6] initially proposed that rat tissues contain a total of four isoenzymes, named A_1, A_2, A_3 and A_4 in the order of their elution, of which A_1 and A_2 are present in submaxillary gland, A_1 and A_3 in liver and A_1 and A_4 in kidney. The major molecular forms reported were A_1 in liver, A_2 in submaxillary gland and A_4 in kidney. However, subsequently they revised the overall number of isoenzymes in rat and human tissues to five with two isoforms each in liver and kidney and a single form in submaxillary gland[7,8]. Other workers[9-12], on the other hand, concluded that the major isoform present in submaxillary gland is identical to that in liver. Unlike Porembska[6] who found two isoenzymes of arginase (A_1 and A_3) in both rat and bovine livers, Tarrab *et al.*[13] described three molecular forms in rat liver while Stewart and Caron[14] could detect only a single form in beef liver. While the basic isoform, A_1, is suggested to function in the urea cycle in rat and human liver[3], the predominant molecular form in bovine liver (A_3) is acidic[6,15]. Spector *et al.*[16,17] observed 25-50% immunoprecipitation of renal arginase by anti-liver arginase antibodies, but other workers[9,10,18-20] failed to detect any cross-reactivity at all between them.

We have been interested in studies on the urea cycle enzymes and characterization of arginase in non-ureotelic animals to understand the metabolic role of arginase outside the urea cycle[21-25]. As a

*Corresponding author.
Present address:
Bioinformatics Center, University of Pune
Pune 411007, India
Tel.: + 91-20-5690195; Fax: + 91-20-5690087
E-mail: reddy@bioinfo.ernet.in

prelude to a comparative study on arginase isoforms in ureotelic and uricotelic vertebrates, we have reinvestigated the molecular forms in rat and beef tissues with a view to reconcile some of the aforementioned anomalies. We made some interesting observations which not only indicate microheterogeneity of the molecular forms, but also show that the isoforms in rat and beef kidneys are immunologically distinct and that a molecular form in rat submaxillary gland is activated by antibodies raised against beef liver arginase. These and other results are reported herein.

Materials and Methods

Sephadex G-100 and G-200 (Pharmacia, Uppsala, Sweden), DEAE-cellulose (C.S.I.R. Center for Biochemicals, New Delhi), CM-cellulose (Whatman Biochemicals, Maidstone, England), beef liver arginase (Serva Feinchemica, Heidelberg, Germany) and Freund's complete adjuvant (Difco Laboratories, Detroit, Michigan, USA) were used. All other chemicals were guaranteed reagent grade products.

Albino rats (Hindustan Antibiotics Ltd., Pimpri, Pune) were dissected under ether anaesthesia to excise liver, kidney and submaxillary gland. Beef liver and kidney were obtained from the local slaughterhouse.

Tissue extracts

Tissue homogenates (10%, w/v) were prepared in chilled 5 m*M* Tris-chloride buffers (*p*H 8.3 and 7.5), both containing 5 m*M* $MnCl_2$, for DEAE- and CM-cellulose chromatographies, respectively. The homogenates were centrifuged (15,000 *g*, 4°C, 15 min) and the supernatants were used for fractionation of isoenzymes.

Fractionation of molecular forms

Fractionation of arginase isoforms was accomplished by ion-exchange chromatography (5°C) on DEAE- and CM-cellulose columns at *p*H 8.3 and 7.5, respectively[5,6]. Aliquots of tissue extracts (5-30 mg protein and 10-150 units of arginase activity; 1 unit=1 μmole product formed per min) were loaded on the columns which were previously equilibrated with 5 m*M* Tris-chloride buffer (*p*H 8.3 or 7.5) and washed with the equilibration buffer till no protein was detected in the fractions (5 ml). The adsorbed forms of the enzyme were eluted with a linear gradient of KCl (0 to 0.3 *M*) in the equilibration buffer. Fractions were collected (flow rate, 20 ml/hr) and monitored for arginase activity, absorbance at 280 nm and conductivity.

Arginase assay, protein estimation and molecular weight determination by gel filtration on Sephadex G-100 or G-200 were carried out as described earlier[26].

Electrophoresis

Anodal and cathodal electrophoreses of arginase were carried out on native polyacrylamide gels (7.5%) in a vertical electrophoresis apparatus with 0.025 *M* Tris-0.192 *M* glycine (*p*H 8.3) and 0.05 *M* Tris-acetate (*p*H 6.5), respectively as tank buffers[27]. After electrophoresis, the gels were sequentially cut into 3 mm slices, each slice was homogenized and assayed for arginase activity as described earlier[23].

Purification of arginase

Arginase was purified from rat liver homogenates by acetone precipitation, heat treatment, ethanol precipitation and CM-cellulose chromatography (*p*H 7.5)[28]. The purified preparation had a specific activity of 540 units per mg protein. Beef liver arginase obtained from Serva Feinchemica (Germany) was further purified by DEAE-cellulose chromatography (*p*H 8.3) to improve the specific activity (30 units per mg protein). Both arginase preparations were found to be homogeneous by electrophoresis on polyacrylamide tube gels under native (see above) and denaturing[29] conditions. Native gel electrophoresis was carried out in the cathodal direction for rat liver arginase and in the anodal direction for beef liver arginase as described above.

Preparation of antibodies

Antisera against purified rat and beef liver arginases were raised in rabbits[30]. Immunoglobulins were purified from the antisera according to Good *et al.*[31]. On native polyacrylamide gel electrophoresis at *p*H 6.5, the immunoglobulins appeared as single well defined bands of cathodal migration. On SDS gels, two bands corresponding to the heavy and light chains of the immunoglobulins were seen by Coomassie Brilliant Blue staining.

Immunotitration

Immunotitration of arginase isoenzymes with different concentrations of purified antibodies raised against rat and beef liver arginases was performed as described[10,32]. Mixtures containing the enzyme preparations and appropriately diluted antibody were adjusted to a final volume of 1 ml with 5 m*M* Tris-chloride buffer (*p*H 7.5) and incubated for 30 min at 37°C. The enzyme-antibody complexes were allowed

to aggregate at 5°C over 18 hr and removed by centrifugation at 13,000 *g* and 4°C for 30 min. Residual arginase activity in the supernatants was assayed.

Results

Fractionation of arginase isoenzymes

DEAE-cellulose chromatography (*p*H 8.3) of tissue extracts yielded in all four fractions of arginase, designated as A_1, A_2, A_3 and A_4 as per the system of nomenclature proposed by Porembska[6]. A_1 emerged out of the column unadsorbed, while A_2, A_3 and A_4 were adsorbed and eluted at 20-70 m*M*, 100-140 m*M* and 190-240 m*M* concentrations, respectively in the KCl gradient. The percent recoveries of arginase activity from the DEAE-cellulose column (mean ± SE) were: rat liver, 67 ± 8; rat submaxillary gland, 82 ± 1; rat kidney, 67 ± 18; beef liver, 99 ± 1; and beef kidney, 48 ± 6.

Arginase from rat liver and submaxillary gland resolved into two peaks each on the DEAE-cellulose column (Fig. 1 A, B). In both cases the major fraction (A_1), containing about 85-96% of the total activity, emerged out of the column unadsorbed (Table 1). The second fraction (A_2) with only about 3-15% activity was eluted by 20-40 m*M* KCl. Re-chromatography of the two fractions under identical conditions gave reproducible elution patterns. Furthermore, the fraction unadsorbed to DEAE-cellulose (A_1) was retained on CM-cellulose (*p*H 7.5) from which it was eluted by 45 m*M* KCl. CM-cellulose chromatography of rat liver and submaxillary gland extracts also showed two forms of arginase, this time with the minor form (A_2) excluded from the column and the major form (A_1), adsorbed to the column, was eluted by 45 m*M* KCl (Fig.1 A, B). Beef liver arginase, on the other hand, eluted from the DEAE-cellulose column as a single peak by 56-65 m*M* KCl corresponding to A_2 (Fig. 1C, Table 1). However, the DEAE-cellulose unadsorbed form (A_1) eluded detection in beef liver extracts.

Chromatography of rat kidney extracts on DEAE-cellulose surprisingly gave four peaks of arginase activity (Fig. 1D). Peak I eluted with the equilibrating buffer and, therefore, appeared to be A_1. The other three peaks were eluted by 60-70 m*M*, 100-120 m*M* and 220-230 m*M* KCl corresponding to A_2, A_3 and A_4, respectively. Thus, all the four forms of arginase appeared to be present in rat kidney. The fractions corresponding to the second and third peaks of activity, which eluted close to each other, were pooled and rechromatographed on DEAE-cellulose to obtain better resolution of A_2 and A_3 (Fig. 1E). Unlike A_1 of rat liver and submaxillary gland, A_1 from rat kidney was not retained on CM-cellulose also (Fig. 1E). Hence this form of arginase excluded by both DEAE-cellulose (*p*H 8.3) and CM-cellulose (*p*H 7.5) was

Table 1—Properties of arginase isoforms in mammalian tissues

[Values given are the mean ± SE]

Tissue	Arginase isoform@	KCl (m*M*)*	% Distribution of activity	K_m(m*M*)	V_{max} (units per mg protein)	Response to #	
						anti-rat liver arginase	anti-beef liver arginase
Rat liver	A_1	0	93 ± 2	41 ± 3	12.75 ± 1.66	–	–
	A_2	23 ± 2	4 ± 1	20 ± 5	0.48 ± 0.15	–	–
Rat submaxillary gland	A_1	0	88 ± 1	15 ± 2	3.55 ± 0.72	–	–
	A_2	37 ± 2	12 ± 2	14 ± 2	0.37 ± 0.07	–	+
Rat kidney	A'_1	0	21 ± 5	18 ± 3	0.13 ± 0.03	0	0
	A_2	65 ± 2	43 ± 2	23 ± 1	0.29 ± 0.12	0	0
	A_3	110 ± 4	26 ± 7	46 ± 10	0.27 ± 0.08	0	0
	A_4	220 ± 3	10 ± 4	24 ± 2	0.19 ± 0.06	0	0
Beef liver	A_2	59 ± 4	100 ± 0	36 ± 6	3.49 ± 0.75	–	–
Beef kidney	A'_1	0	48 ± 8	17 ± 4	0.21 ± 0.02	–	–
	A_2	56 ± 1	52 ± 8	50 ± 4	0.16 ± 0.04	–	–

@A_1 is adsorbed to CM-cellulose (*p*H 7.5) but not to DEAE-cellulose (*p*H 8.3); A'_1 is not adsorbed to both CM- and DEAE- celluloses

* Concentration required for elution from DEAE-cellulose (*p*H 8.3)

\# Precipitation (–); no cross-reactivity (0); activation (+)

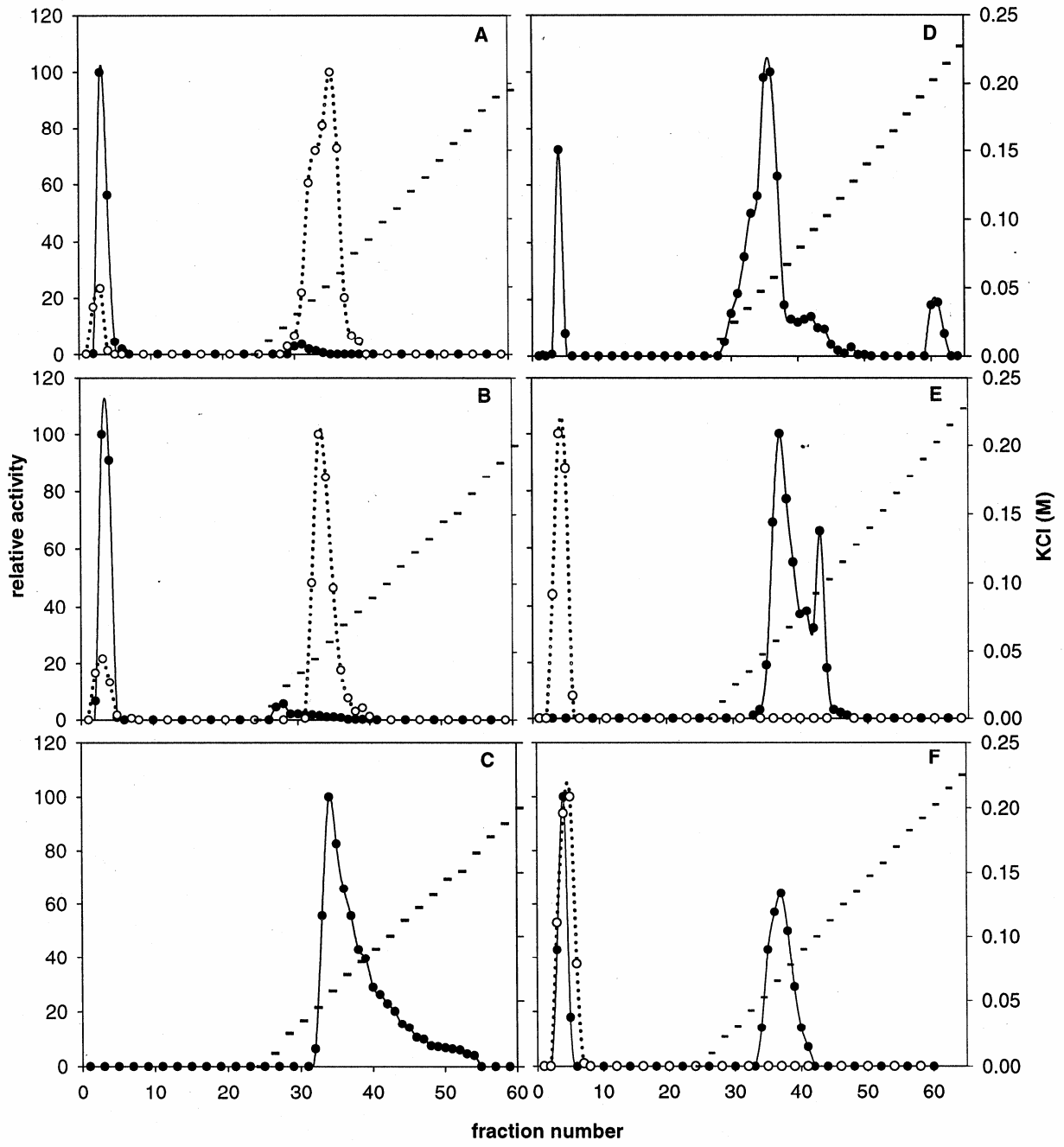

Fig. 1—Fractionation of arginase isoforms: [(A), rat liver; (B), rat submaxillary gland; (C), beef liver; (D & E), rat kidney; and (F), beef kidney. Tissue extracts were fractionated by DEAE-cellulose, *p*H 8.3; (•—•) and CM-cellulose, *p*H 7.5; (o….o) chromatography. KCl gradient used for eluting the adsorbed isoforms is indicated (–). Pooled fractions corresponding to isoforms A_2 and A_3 (peaks 2 and 3) in Fig. D were rechromatographed on DEAE-cellulose to achieve better resolution (E). DEAE-cellulose unadsorbed isoforms in rat kidney (D) and beef kidney (F) were excluded by CM-cellulose on rechromatography (E, F)]

designated as A'_1 as suggested by Konarska *et al.*[32] for a similar molecular form present in human fibroblasts and foetal calf serum. Of the four forms, A_2 was quantitatively the most predominant and A_4 the least (Table 1).

Arginase in beef kidney homogenates, on the other hand, separated only into two peaks on DEAE-cellulose, of which the first was unadsorbed and the second eluted by 55-60 m*M* KCl, corresponding to A_1 and A_2 respectively (Fig. 1F). A_1 in beef kidney, like

that in rat kidney, was also excluded by CM-cellulose (Fig. 1F) and hence appeared to be A'_1. In general, the two molecular forms were present more or less in equal proportions in beef kidney (Table 1).

Native gel electrophoresis

The electrophoretic behaviour of the fractionated forms of arginase on native polyacrylamide gels was consistent with their chromatographic behaviour. A_1 from rat liver and submaxillary gland proved to be distinctly cationic during electrophoresis at *p*H 6.5. A'_1 from the kidney of the two mammalian species appeared to be less cationic, migrating to the cathode at *p*H 6.5 and to the anode at *p*H 8.3. A_2, A_3 and A_4 were all anionic at *p*H 8.3 and the electrophoretic mobility of the four renal forms of the rat to the anode (*p*H 8.3) increased in the order, $A'_1<A_2<A_3<A_4$ (Fig. 2).

Physical and kinetic properties

No significant differences were found in the molecular weights (110,000-128,000) and *p*H optima (9.4-10.1) of the different molecular forms. The apparent K_m values of the isoforms (14-50 m*M*, Table 1), though relatively high, were comparable to those (20-40 m*M*) reported by Mora *et al.*[33] for liver arginase from different ureotelic vertebrates and did not show any discernible systematic differences. Except for A'_1 from rat kidney and A_2 from rat liver and submaxillary gland, which showed mixed type inhibition, all other isoforms were inhibited competitively by 5 m*M* L-ornithine and L-lysine. Mixed type inhibition of rat liver A_2 and kidney A'_1 by the two amino acids was associated with a greater change in the apparent K_m (150-180% increase) than in the V_{max} (15-35% decrease). With rat submaxillary gland A_2, on the other hand, both parameters changed by about the same extent (25-30%) in the presence of the inhibitory amino acids.

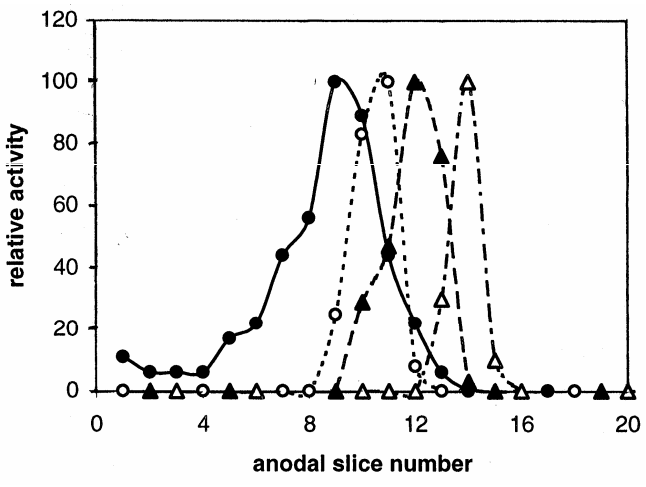

Fig. 2—Anodal electrophoresis (*p*H 8.3) of rat kidney isoforms [A'_1, (•—•); A_2, (o---o); A_3, (▲---▲); and A_4, (Δ---Δ); Isoforms fractionated by DEAE-cellulose chromatography (Fig. 1D) were subjected to electrophoresis simultaneously on separate polyacrylamide tube gels. Tube gels were sliced and arginase activity in the slices was determined]

Immunological characterization

All the molecular forms of arginase were treated with different concentrations of purified anti-rat liver (0-70 mg per unit enzyme) and anti-beef liver (0-24 mg per unit enzyme) arginase antibodies and residual enzyme activities were assayed in the supernatants after removing the antigen-antibody complexes by centrifugation. Molecular forms A_1 and A_2 from rat liver and submaxillary gland as well as A_2 from beef liver were all progressively precipitated with increasing concentration of anti-rat liver arginase (Fig. 3A). The immunotitration profiles of the two isoforms, A_1 and A_2, from rat liver and submaxillary gland were rather similar. Beef liver A_2 was also completely precipitated by anti-rat liver arginase, but only at a concentration 3,500-fold higher than that required for the total precipitation of rat liver A_1.

Rat liver A_1 and A_2 were precipitated to the extent of 80% by anti-beef liver arginase at a concentration 4-fold higher than that required for completely precipitating the homologous enzyme (Fig. 3B). Rat submaxillary gland A_1, on the other hand, was only precipitated by about 20% at this concentration of the antibody. Interestingly, however, rat submaxillary gland A_2 was activated by anti-beef liver arginase, as indicated by the 3.5-fold increase of enzyme activity in the supernatant (Fig. 3B).

None of the molecular forms present in rat kidney was precipitated by anti-rat liver arginase even at concentrations 800-1000 fold higher than that required for the precipitation of the homologous antigen (Fig. 4A). Anti-beef liver arginase antibodies were also totally ineffective in precipitating these isoforms at concentrations 10-fold higher than that causing complete precipitation of beef liver A_2 (Fig. 4B). Both isoforms present in the beef kidney, however, were immunoprecipitated by 50-70% at lower concentrations of the two antibodies (Fig. 4 A, B).

The immunoprecipitates of A_1 and A_2 from rat liver and submaxillary gland with anti-rat liver arginase as

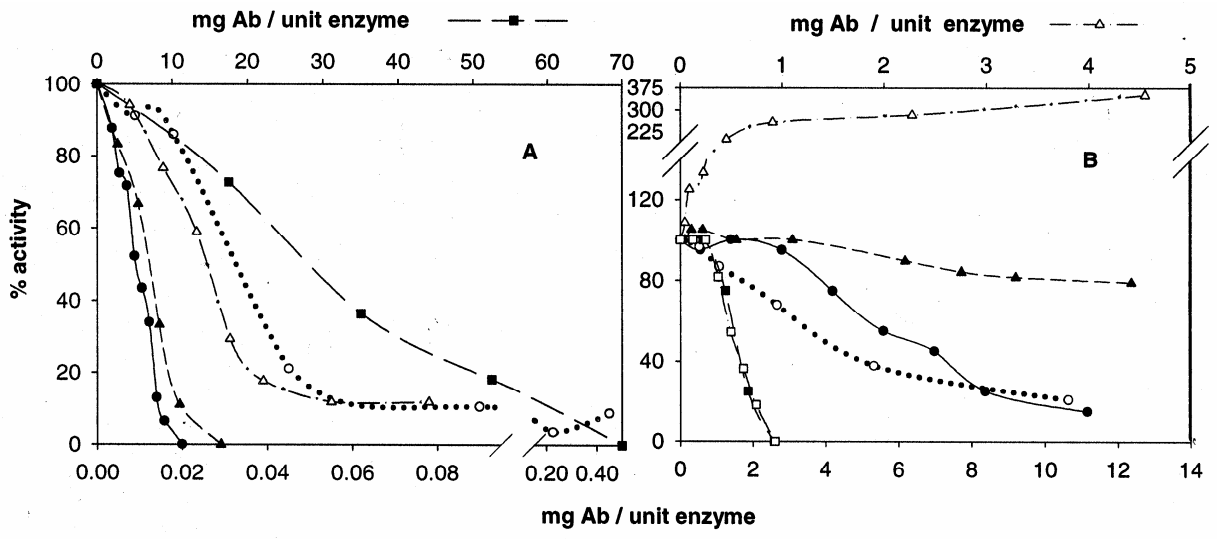

Fig. 3—Immunotitration profiles of arginase isoforms from rat liver, rat submaxillary gland and beef liver with (A) anti-rat liver arginase and (B) anti-beef liver arginase antibodies: [rat liver A_1, (•—•); and A_2, (o….o); submaxillary gland A_1, (▲--▲); and A_2, (Δ--Δ;); beef liver A_2, (■--■) and purified beef liver arginase from Serva Feinchemica, (□-..--□). Antigen-antibody mixtures were centrifuged and the percent residual activity in the supernatants determined. The upper scales on the X-axis in Fig. A (0 to 70 mg Ab per unit enzyme) and Fig. B (0 to 5 mg Ab per unit enzyme) are for beef liver A_2 and rat submaxillary gland A_2 respectively. The lower scales on X-axis in both Figs. are for all other isoforms]

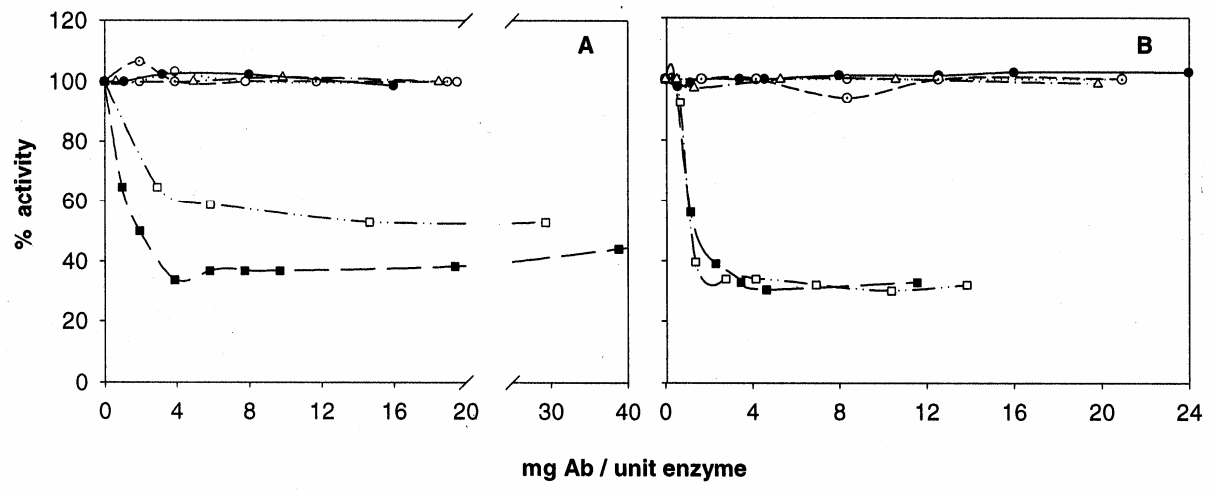

Fig. 4—Immunotitration of renal arginase isoforms with (A) anti-rat liver arginase and (B) anti-beef liver arginase antibodies: [rat kidney A'_1, (•---•); A_2, (o---o); A_3, (ʊ---ʊ); and A_4, (Δ---Δ); and beef kidney A'_1, (■-.-■); and A_2, (□-..-□). The percent residual activities in supernatants obtained by centrifugation of antigen-antibody mixtures were plotted]

well as those of A_2 from beef liver and A'_1 and A_2 from beef kidney with anti-beef liver arginase were visualized by Ouchterlony double immunodiffusion and immunoelectrophoresis. However, no preciptin bands or arcs could be detected with any of the rat kidney isoforms (A'_1, A_2, A_3 and A_4) and anti-rat liver arginase by either technic.

Discussion

Our experiments have indicated a total of four, and not five, molecular forms of arginase in rat and beef tissues in accordance with the initial observations of Porembska[6]. The existence of four isoenzymes in all and two in a given tissue is clearly consistent with the currently accepted concepts of two genes[4] and

trimeric structure[34,35] of arginase. Despite the substantial differences in their chromatographic behaviour and immunological cross-reactivity, the isoforms are remarkably similar in their molecular size, substrate affinity, *p*H optimum and inhibition by L-ornithine and L-lysine. The variation in charge and antigenicity has apparently been accomplished by changes in composition of the enzyme molecules in regions away from the active site[3].

The same pair of isoenzymes, A_1 and A_2, are present in rat liver and submaxillary gland. This conclusion, based on chromatographic evidence, is supported by the similarity in the immunoprecipitation profiles of the isoforms in the two tissues by anti-rat liver arginase antibodies. Furthermore, A_1 is the major isoenzyme representing about 85-96% of the total arginase in both tissues. Though in sharp contrast with the results of Porembska[6] that A_1 and A_2 are respectively the major molecular forms in rat liver and submaxillary gland, our findings are in agreement with the conclusion of other workers[9–11] that the isoenzyme of arginase present in submaxillary gland is identical to that in liver. Based on Western and Northern blot analyses of arginase I and II proteins and their mRNAs, Akibo *et al.*[12] have confirmed that, like in liver, AI (A_1) is the major molecular form in salivary glands.

The responses of A_2 in rat liver and submaxillary gland to anti-beef liver arginase antibodies are different. Both A_1 and A_2 in rat liver are immunoprecipitated by about 80% in the presence of these antibodies. While A_1 in submaxillary gland is slightly precipitated (20%), A_2 is actually activated 3.5-fold. Stimulation of enzyme activity in the presence of antibodies reflects the failure of the enzyme-antibody complexes to form precipitable aggregates and antibody-induced conformational changes that influence the properties of the active site[36]. Although the structural features contributing to the activation of submaxillary gland A_2 by beef liver arginase antibodies are not apparent, augmentation of enzyme activity by specific antibody molecules is known for bacterial α-amylase[37], penicillinase[36] and β-galactosidase[38,39], bovine pancreatic ribonuclease[40] and monkey liver phenylalanine hydroxylase[41].

The occurrence of a single species of arginase, corresponding to A_2, in beef liver is in conformity with the observation of Stewart and Caron[14] who also reported a solitary form of arginase that does not bind to CM-cellulose (*p*H 7.0) in this tissue. However, Porembska[6], found two isoenzymes, A_1 and A_3, in the ratio of 13:87 in ox liver. While A_1 might have escaped detection due to its presence in trace quantities, the second isoform in our experiments with beef liver is A_2 and not A_3. Turkoglu and Ozer[42] reported that bovine liver arginase, which appears as a single component in Matrix Gel Red A chromatography, resolves into three peaks of distinct *p*I values on chromatofocusing, suggesting that some of these differences might be due to microheterogeneity that manifests under different experimental conditions. The predominance of DEAE-cellulose (*p*H 8.3) unadsorbed (A_1) and adsorbed (A_2) forms in rat and beef livers respectively, is consistent with the isoelectric points reported for arginases in these tissues[15,43]. The precipitation of rat and beef liver arginases by antibodies raised against either enzyme is in keeping with the immunological cross-reactivity known for hepatic arginases from different mammals[20, 44].

The apparent resolution of rat kidney arginase into four peaks, corresponding to A'_1, A_2, A_3 and A_4, on DEAE-cellulose (Fig. 1D) is surprising because: (i), only two isoforms of arginase have been hitherto described in this tissue[5,10,45-47]; and (ii), we ourselves could detect only two molecular forms, A'_1 and A_2, in approx. equal proportions in beef kidney (Fig. 1F). According to Porembska[6], however, the kidney in all mammalian species contains two isoenzymes, A_1 and A_4, the latter accounting for 88-97% of total renal arginase. In the absence of any compelling physiological reasons for the requirement of additional isoenzymes in rat kidney, A_3 and A_4 in this tissue are probably expressions of charge microheterogeneity of the type reported earlier for mouse and beef liver arginases[42,48]. The reasons for this apparent microheterogeneity of the molecular forms in rat kidney are not clear. Since all the four molecular forms in this tissue have the same molecular weight (118,000), A_3 and A_4 could not have arisen from the partial endogenous proteolysis of A_2, A_1 or both.

None of the four isoforms in rat kidney showed any cross-reactivity, whatsoever, with anti-rat liver and anti-beef liver arginase antibodies. Complete immunological incompatibility between unfractionated arginase preparations from rat liver and kidney as well as the major isoforms isolated from the two tissues has been earlier demonstrated[7–10,18,49]. Spector *et al.*[16,17], on the other hand, observed 25-50%

immunoprecipitation of rat and human kidney arginase by antibodies to the liver enzyme from the same species. While antibodies raised against A_1 from rat and human liver have been reported to specifically immunoprecipitate only A_1, but not the second isoform, in the kidney of the two species[19,32,50], none of the isoforms in rat kidney is even partially precipitated by either antibody in our experiments. Both isoforms in beef kidney, unlike those in rat kidney, cross-reacted with the two antibodies resulting in 50-70% precipitation. It appears paradoxical that anti-rat liver arginase antibodies cross-react with the isoforms in beef kidney, but not with those in rat kidney.

Ignoring the enigmatic occurrence of A_3 and A_4 in rat kidney as artifactitious, it can be concluded that the other mammalian tissues examined here contain two isoenzymes of arginase, A_1 and A_2. The only exception is beef liver, where A_1 is below the levels of detection. A_1 is a basic form excluded by DEAE-cellulose (*p*H 8.3), but retained on CM-cellulose (*p*H 7.5). A_2 is a neutral or slightly acidic form which is retained on the former and excluded by the latter. The two isoforms exhibit microheterogeneity so that A_1 or A_2 present in one tissue is not necessarily identical to that in another tissue. In rat and beef kidneys, A_1 is replaced by its variant A'_1 which is excluded by both ion-exchangers. While A_1 in rat liver and submaxillary gland as well as A'_1 in beef kidney are all immunoprecipitated either *in toto* or in part by both anti-rat liver and anti-beef liver arginase antibodies, A'_1 in rat kidney is not precipitated at all by either antibody.

The heterogeneity of A_2 is evident from the fact that the isoform in rat liver and submaxillary gland is eluted by a lower concentration of KCl (23-37 m*M*) than that in beef kidney, beef liver and rat kidney (56-65 m*M*, Table 1). Moreover, A_2 from rat liver, beef liver and beef kidney is precipitated by both antibodies, but neither antibody cross-reacts with A_2 in rat kidney. Rat submaxillary gland A_2, on the other hand, is slightly precipitated by anti-rat liver arginase, but substantially activated by anti-beef liver arginase. The immunological evidence suggests that rat submaxillary gland A_1 is antigenically the closest and the molecular forms in rat kidney are the farthest to rat liver arginase A_1. Similarly, beef kidney A_1 and A_2 appear to be the closest and the isoforms in rat kidney the farthest to beef liver arginase A_2. Since the hepatic (type I) and extrahepatic (type II) forms of arginase are believed to be coded by two different genes[3,4], the chromatographic and immunological microheterogeneity of A_1 and A_2 in mammalian tissues is possibly a reflection of post-translational modifications[51]. However, it may be mentioned here that three almost identical non-hepatic arginase genes have been cloned in *Xenopus laevis*[52].

Different metabolic functions have been assigned to the two isoforms of arginase. While type I (AI) arginase is said to catalyze the last step in the urea cycle in liver, type II (AII) enzyme is suggested to generate ornithine as a precursor for the synthesis of proline, glutamate and polyamines in extrahepatic tissues[2-4]. Grody *et al.*[3] have equated type I arginase with A_1 and type II with A_4 of Porembska[6]. However, the absence of A_4 in beef kidney and the presence of A_2 in all tissues prompts us to propose that A_2 in all probability represents type II arginase. A_1, which is suggested to function as a component of the urea cycle in rat and human liver, is absent in beef liver, where A_2 presumably functions in that capacity. Furthermore, A_1 is the major isoenzyme of arginase in salivary glands, where the enzyme has been implicated in the formation of proline, glutamate and polyamines[12], a function attributed to type II arginase in other extrahepatic tissues like kidney, brain and intestine[53–55]. In conclusion, it appears that different isoforms of arginase possibly serve as components of the urea cycle in the liver of different mammals or of the catabolism of arginine to proline, glutamate and polyamines in different extrahepatic tissues of a given mammal.

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