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ARGINASE IN BULL TESTIS

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Bull testis arginase was about 1 000 times purified. The molecular weight was estimated to 120 000 (±250). The enzyme was an anionic protein. K_m was determined to be 3.4 mM. According to electrophoretic mobility and affinity to DEAE-cellulose as well as to the immunological properties, the arginase isolated from bull testis may be identified as arginase A_1.

Arginase (L-arginine amidinohydrolase, EC 3.5.3.1), one of the urea cycle enzyme, catalyses in mammalian liver the cleavage of L-arginine to urea and ornithine. The enzyme is also present in other tissues where it is involved in polyamine or proline biosynthesis [1, 2].

The presence of different molecular forms of arginase in mammalian tissues has been postulated by many authors [3 - 6].

Studies on the properties of arginase from human tissues performed in our laboratory have revealed the presence of five enzyme forms [7]. On the basis of electrophoretic mobilities, immunological responses and different isoelectric points these forms of arginases were designated: A_1, A_2, A_3, A_4 and A_5.

So far, little attention has been paid to the presence of arginase in reproductive organs.

The fact that arginase occurs in various molecular forms, which may performs several functions in various tissues led us to attempt isolation and characterization of this enzyme from bull testis.

MATERIALS AND METHODS

Reagents. Reagents were purchased as follows: L-arginine (Calbiochem, Los Angeles, CA), Sephadex G-100, Sephadex G-150 and Dextran 2 000 (Pharmacia, Uppsala, Sweden), DEAE-cellulose (DE-11) (Whatman Biochemicals, Madison, Kent, England). Freund's incomplete and complete adjuvant (Difco Laboratories, Detroit, Mi).

Bull testis. Testes were obtained from a local abattoir immediately after slaughter of animals. The organ was dissected, cleaned of fat and stored at −30°C for later use.

Tissue homogenization. The testis was minced with scissors and homogenized in 0.1 M Tris/HCl buffer, pH 7.5, containing 0.1% (v/v) of Triton X-100 (300 mg tissue per 1 ml of buffer), in a glass homogenizer and with a teflon pestle. The homogenate was then centrifuged at $30000 \times g$ for 20 min, and used for enzyme activity determination or further purification.

Arginase assay. The enzymatic activity was determined according to Chinard [8].

Protein determination. Protein was assayed according to Lowry *et al.* [9] with crystalline serum albumin as a standard.

Molecular weight determinations. The molecular weight of bull testis arginase was determined by Sephadex G-150 chromatography, according to Andrews [10]. The column (40 × 2 cm) was first equilibrated with 100 mM KCl in 50 mM Tris/HCl buffer, pH 7.5.

Horse myoglobin (M_r 17000), ovalbumin (M_r 46000), bovine serum albumin (M_r 69000) and bovine globulin (M_r 150000) were used as standards. Fractions of 2 ml were checked for enzymic activity and protein content.

Electrophoresis. The assay was performed according to Laemmli [11] in 7.5% polyacrylamide gel, pH 8.9, for 90 min at 9 V/cm. After electrophoresis protein was stained with 0.5% Amido Black solution in 7% acetic acid.

Double immunodiffusion. The test was performed according to Ouchterlony [12] at room temperature for 48 h; the plates were washed with saline and stained with 0.1% Coomassie blue R-250.

Antiserum. Pure arginase from bull testis, as well as pure arginase A_1 from human kidney and A_5 from human liver, were injected into female guinea pig. The animals were immunized every 10 days with 200 μg of arginase in 0.5 ml of saline supplemented with 0.5 ml of Freund's incomplete (first time) and complete (subsequent immunizations) adjuvant. Animals were bled after 6 weeks from the first immunization and blood was centrifuged at $10000 \times g$ for 30 min in a Sorvall RC 2-B SS-34 rotor.

Serum protein was precipitated by addition of 10 g of ammonium sulphate per 20 ml of serum, centrifuged at $15000 \times g$ for 30 min, dissolved in a small volume of saline and dialysed against 3 litres of saline for 48 h.

Pure arginase A_1 from human kidney and pure arginase A_5 from human liver were obtained as reported by Zamecka & Porembska [7].

RESULTS

Procedure for purification of bull testis arginase

Among the three reagents tested for the efficiency of arginase extraction from bull testis 0.25% Triton X-100 was found to give the best yield (Table 1).

The enzymic activity in the latter extract was 0.43 µmoles ornithine/min per g tissue.

During the purification procedure, 1 mM $MnCl_2$ and 1 mM 2-mercapto-ethanol were present in buffers to stabilize the enzyme. The purification was carried out at 4°C as reported by Kedra-Luboińska *et al.* [13].

Table 1
Extraction of bull testis arginase

Procedure	Protein mg/ml	Activity	
		Specific activity*	Units/g tissue
0.1 M KCl	1.4	0.010	0.09
0.25% Tween 80	3.5	0.013	0.22
0.25% Triton X-100	5.4	0.020	0.43

* µmole of ornithine · min^{-1} per mg protein.

Properties of enzyme

Electrophoretic properties. When bull testis arginase was subjected to polyacrylamide gel electrophoresis at pH 8.9, only a single protein band containing arginase activity was observed (Fig. 1b). Bull testis arginase is an anionic protein whose electrophoretic mobility is not identical, but closely

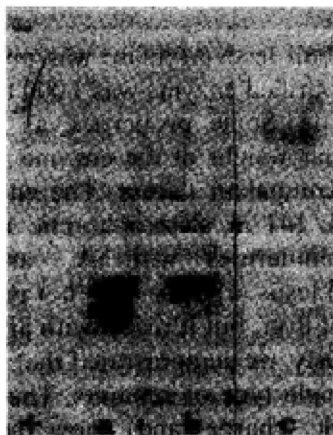

Fig. 1. Electrophoretic pattern of bull testis arginase. Polyacrylamide gel electrophoresis was performed as described in Material and Methods. a, Mixture of pure human kidney arginase A_1 (30 µg) plus bull testis enzyme after 6th step purification (25 µg); b, bull testis arginase after 6th step purification (25 µg); c, pure human liver arginase A_5 (30 µg)

approaches the mobility of arginase A_1 from human kidney (Fig. 1a, b). The charge of bull testis enzyme differs greatly from that of arginase A_5 from human liver (Fig. 1c).

Molecular weight. The M_r of bull testis arginase was found to be $120\,000 \pm 2\,500$ (Fig. 2).

Optimum pH. For bull testis arginase, similarly as for other mammalian arginases, the optimum pH was 9.5.

Substrate specificity. Bull testis arginase was highly specific for L-arginine; it did not hydrolyse D-arginine, L-homoarginine, or any of the tested guanidine derivatives: β-guanidine-propionate, 1-α-amino-β-guanidinepropionate, γ-guanidinebutyrate and α-amino-γ-guanidinebutyrate.

Effect of substrate concentration. Enzyme activity was measured in a mixture containing 10 mM barbituric buffer, pH 9.5 and 1.0 mM $MnCl_2$. The K_m value was determined by the graphic method of Lineweaver-Burke. For bull testis arginase it was calculated to be 3.4 mM (Fig. 3).

Immunological properties. Antisera were used to evaluate differences and similarities between bull testis arginase and parental forms A_1 from human kidney as well as parental form A_5 from human liver [7]. In the double immunodiffusion test, arginase from testis gave a crossreaction with its own antiserum (Fig. 4a). The enzyme reacted also with antiserum against pure arginase A_1 from human kidney (Fig. 5a). However, when the testis enzyme was compared with the arginase from human kidney, the presence of a spur, testifing to only partial immunological identity, was always found (Fig. 5b). Arginase from bull testis gave no crossreaction with antiserum against arginase A_5 from human liver.

DISCUSSION

In the present studies, bull testis arginase was purified and characterized. The specific activity of the purified enzyme was 1 000 times greater than that of the crude testis homogenate. Some properties, as optimum pH, substrate specificity, K_m and molecular weight of the enzyme resembles those of other arginases from different mammalian tissues. The enzyme from bull testis is similar to arginase A_1 [7, 14] in electrophoretic mobility and affinity to DEAE-cellulose. Close similarities with A_1 are also confirmed by immunological studies. Bull testis arginase does not precipitate with antiserum against arginase A_5 from rat liver, but it reacts with antiserum against arginase A_1 from rat kidney. Previously, we demonstrated that both arginase A_1 and A_5 were build up each of a single type of subunits. The subunits of these forms differ however, in electric charge and show complete immunological incompatibility [7]. Probably arginase from bull testis is built of subunits of a single kind, i.e. of form A_1 subunits, because it does not react with antiserum against form A_5. It should be noted that oligomeric forms A_2, A_3, A_4 which

Table 2
Purification of bull testis arginase

Procedure	Total protein (mg)	Activity		Purification factor
		Total units	Specific* activity	
Extract of bull testis	12 000	240	0.02	—
0.45-0.75 $(NH_4)_2SO_4$ saturation	4 600	230	0.05	2.5
Heating at 55°C	800	152	0.192	9.6
Ethanol precipitation	300	114	0.384	19.2
DEAE-cellulose chromatography	40	76	1.9	95
Sephadex G-150 filtration	7	65	9.5	475
DEAE-Sephacel chromatography	3	60	20.0	1 000

* µmoles of ornithine · min^{-1} per mg protein.

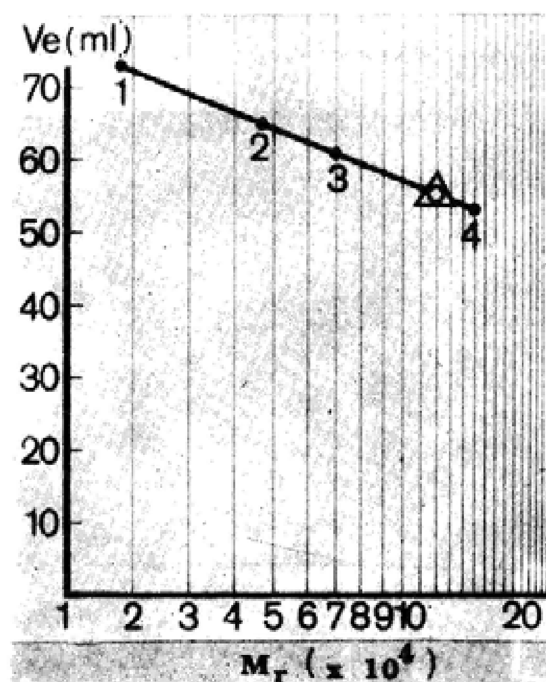

Fig. 2. Molecular weight determination of bull testis arginase by Sephadex G-150 filtration. The enzyme obtained after step 5 and marker proteins (6 mg each) were applied. 1, horse myoglobin, M_r 17 000; 2, chicken egg albumin, 45 000; 3, bovine serum albumin, 69 000; 4, bovine serum globulin, 150 000; ○, bull testis arginase

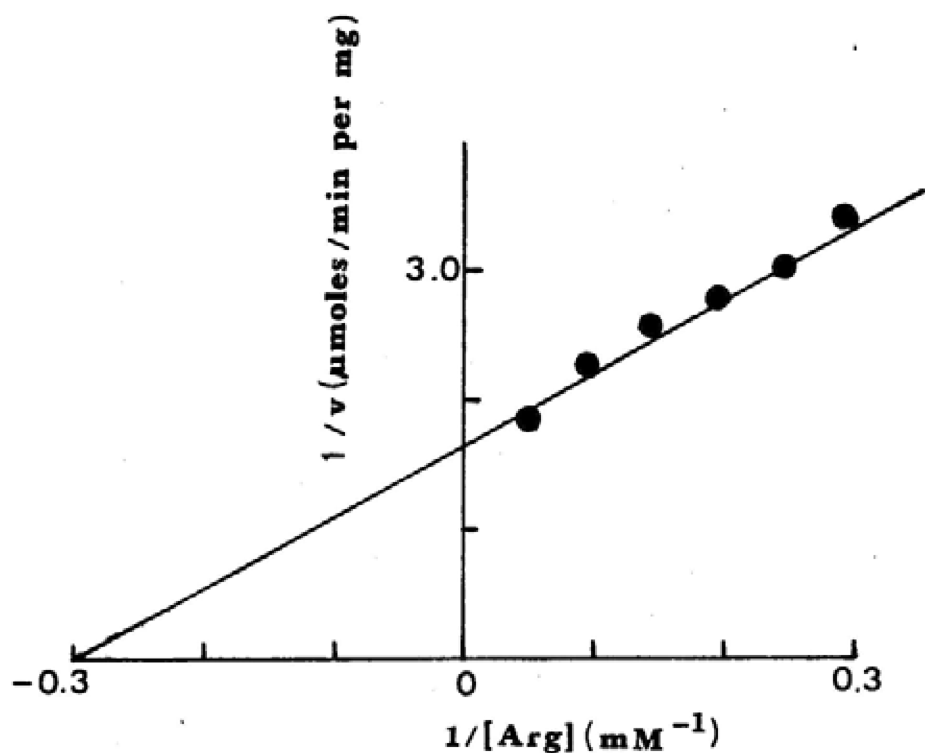

Fig. 3. Lineweaver-Burk plot of bull testis arginase (8 μg of enzyme obtained after step 6 was used)

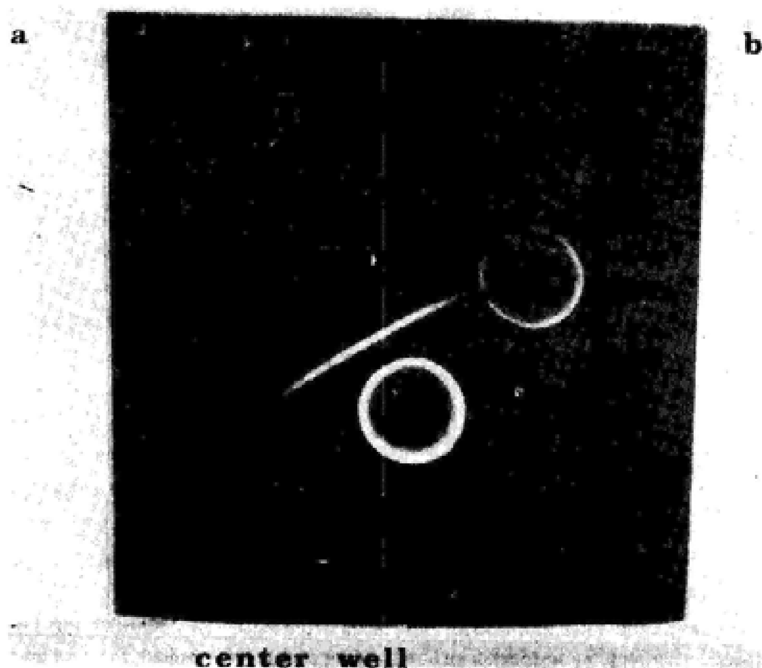

Fig. 4. Ouchterlony double-immunodiffusion test of bull testis arginase. Center well: pure bull testis arginase; a, antiserum against bull testis arginase; b, A_5 arginase

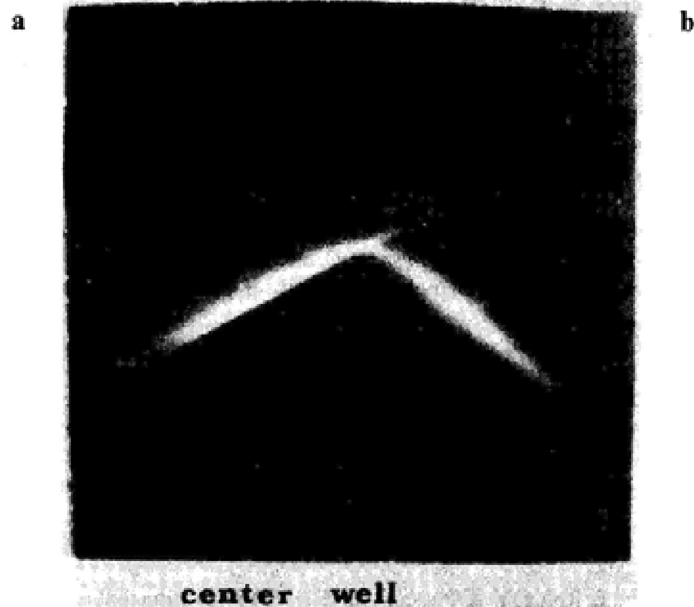

Fig. 5. Cross-reactivity of bull testis arginase and arginase A_1 from human kidney in the presence of antiserum against form arginase A_1 of human kidney center well: antiserum against arginase A_1 from human kidney; a, arginase A_1 from human kidney; b, pure bull testis arginase

comprise subunits of both types and display partial immunological similarity with respect to each other and to arginase A_1 and A_5 [7], react with antiserum against form A_5.

The physiological function of bull testis arginase is far from being elucidated. This form of arginase has been shown to be present also in rat kidney [15], intestine [16] and brain [17]. It has been postulated [15, 18] that rat kidney arginase is a mitochondrial enzyme whereas arginase from rat liver is a cytosolic one. It has been postulated that kidney arginase participates in proline and polyamine metabolism [1, 2].

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