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IMMUNOLOGICAL PROPERTIES OF RAT ARGINASES

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This paper is dedicated to the memory of Professor Józef Heller

Five immunologically different forms of arginase were evidenced in rat tissues by the double diffusion test and immunoelectrophoresis. New symbols for these arginases are proposed (beginning with the most anionic forms): A1 (kidney), A2 (liver), A3 (salarvary glands), A4 (kidney) and A5 (liver).

Arginases A1 from kidney and A5 from liver are paternal forms built of one-type subunits. Subunits of form A1 exhibit a non-identity cross-reaction with subunits of form A5.

Arginases A2, A3 and A4 are hybrids composed of both kinds of subunits.

Arginase A1 from rat liver and arginase A4 from rat kidney are oligomeric proteins. Each form is composed of identical subunits of Mr 30 000 (Skrzypek-Osiecka et al., 1983) but since arginase A1 consists of cationic and arginase A4 of anionic protein chains (Skrzypek-Osiecka et al., 1983) their subunits differ considerably in electric charge. Recombination in vitro of these two types of subunits gives five forms of arginase: two paternal forms and three hybrids (Skrzypek-Osiecka & Porembska, 1983). All five recombinants have the same molecular weight (120 000) but differ in electrophoretic and immunological properties.

At the earlier stages of our research (Gąsiorowska et al., 1970) four forms of arginases (A1, A2, A3, A4) have been detected in rat tissues; recently, preliminary evidence was obtained for non-identity of liver arginase A1 and kidney A1 (Skrzypek-Osiecka et al., 1983).

It is therefore necessary to verify the number of arginase forms in rat tissues, and this is the objective of the present work.
MATERIALS AND METHODS

Reagents. All reagents were the same as reported by Skrzypek-Osiecka et al. (1983).

Animals. Male Wistar rats weighing 150 g were used. The liver, kidney and salivary glands were isolated, washed with 0.9\% NaCl and used for preparation of various forms of arginase. In the experiments the following preparations were used: pure arginase A_1 from rat liver, A_4 from rat kidney and partly purified preparations of A_4 from rat kidney and rat salivary glands, A_2 from rat salivary glands, and A_3 from rat liver. The arginase forms were designated according to Gasiorkowska et al. (1970), and this nomenclature was used throughout this paper. Pure homogeneous arginase A_1 from liver (spec. act. 3500 nmol \cdot min^{-1} \cdot mg^{-1} protein) was obtained according to Ber & Muszyńska (1979), arginase A_4 from kidney (spec. act. 1050 nmol \cdot min^{-1} \cdot mg^{-1} protein), and partly purified arginase A_4 from kidney (spec. act. 40 nmol \cdot min^{-1} \cdot mg^{-1} protein) were prepared as reported by Skrzypek-Osiecka et al. (1983). To obtain partly purified arginase from salivary glands (spec. act. 30 nmol \cdot min^{-1} \cdot mg^{-1} protein) and arginase A_3 from liver (spec. act. 32 nmol \cdot min^{-1} \cdot mg^{-1} protein), ammonium sulphate precipitation, acetone fractionation, heat treatment, as well as CM- and DEAE-cellulose chromatography were applied according to Skrzypek-Osiecka et al. (1983). Each preparation represented only one form of arginase of M, 120 000.

Double immunodiffusion. The test was carried out according to Ouchterlony (1967) in 1\% agar containing 0.01 m-veronal buffer, pH 8.1. Agar slides were kept at room temperature for 48 h, whereupon they were washed successively with saline and water, dried and stained with 0.1\% Coomassie Blue R 250.

Immunoelectrophoresis. The assay was performed at 8°C in agarose gel containing 0.02 m-veronal buffer, pH 8.6, for 90 min at 9 V/cm. After electrophoresis appropriate antibodies were applied and following 48 h incubation at room temperature the slides were washed successively with saline and water, dried and stained with 0.1\% Comassie Blue R 250.

Antibodies. Guinea pigs were immunized with high-purity arginase A_1 from rat liver and high-purity arginase A_4 from rat kidney, respectively. In the course of 4 weeks, guinea pigs received four injections of 200 - 300 mg of arginase A_1 and A_4, respectively, in 0.1 ml Freund’s adjuvant. After approximately 5 weeks the animals were bled through cardiac puncture. The antisera obtained were stored at −18°C.

Conditions of dissociation into subunits. Dissociation of native arginases was carried out in the presence of EDTA (Skrzypek-Osiecka et al., 1983).

RESULTS

In the double immunodiffusion test, arginases A_1 and A_2 from rat liver, A_3 from kidney and A_2 from rat salivary glands reacted with antibodies against arginase A_1 from rat liver (Fig. 1), whereas there was no cross-reaction with arginase A_4 from rat kidney (Fig. 1A, IVb).
Fig. 1. Immunodiffusion of guinea pig antiserum against arginase A₁ from rat liver.
A, The relationship of A₁ form from liver with other arginase forms. Center well, antiserum against liver arginase A₁; Ia, IIa, IIIa and IVa, liver arginase A₁; Ib, kidney A₁; IIb, A₂ from salivary glands; IIIb, liver A₃; IVb, kidney A₄.
B, Cross-reactivity of rat arginases. Center well, antiserum against liver arginase A₁; Ia, liver A₁; Ib, kidney A₁; IIa, kidney A₁; IIb, A₂ from salivary glands; IIIa, A₂ from salivary glands; IIIb, liver A₃.
Fig. 3. Cross-reactivity of liver arginase A₁ and kidney arginase A₄ and their subunits in the presence of the antisera against liver A₁ and kidney A₄ arginases. Center well: I, arginase A₁ and arginase A₄; II, EDTA-treated arginase A₁ and arginase A₄; a, antiserum against liver arginase A₁; b, antiserum against kidney arginase A₄.
Fig. 4, Immunoelectrophoresis of EDTA-treated arginases. Subunits of: kidney arginase $A_4$ (a); arginase $A_2$ from salivary glands (b); arginase $A_1$ from liver (c). Cross-reaction developed with the antiserum against liver arginase $A_1$ and kidney arginase $A_4$. 
Fig. 5. Immunoelectrophoresis of native forms of arginases and EDTA-treated preparation of arginase A₂ from salivary glands. a, Kidney arginase A₄; b, EDTA-treated arginase A₂ from salivary glands; c, liver arginase A₁; d, arginase A₂ from salivary glands. Cross-reaction as in Fig. 4.
Fig. 6. Immunoelectrophoresis of rat arginase forms: a, $A_4$ from kidney; b, $A_3$ from liver; c, $A_2$ from salivary glands; d, $A_1$ from kidney; e, $A_1$ from liver. Cross-reaction developed in the presence of antiserum against arginase $A_1$ from liver (I) and antiserum against arginase $A_4$ from kidney (II).
Fig. 2. Immunodiffusion of guinea pig antiserum against arginase A4 from rat kidney.
A, Relationships of A4 form from kidney with the other forms of arginases. Center well, antiserum against arginase A4 from kidney; Ia, IIa, IIIa, and IVa, kidney arginase A4; Ib, liver A3; IIb, A2 from salivary glands; IIIb, kidney A1; IVb, liver A1.
B, Cross-reactivity of rat arginases. Center well, antiserum against arginase A4; Ia, kidney A4; Ib, liver A3; IIa, liver A3; IIb, A2 from salivary glands; IIIa, A2 from salivary glands; IIIb, kidney A1.
Partial identity was detected between liver arginase A₁ and the other three forms of arginases. Each slide exhibited a spur indicating that some antigenic determinants of arginase A₁ from liver were absent in arginase A₁ from kidney, A₂ from salivary glands and A₃ from liver. These forms did not show antigenic identity with each other (Fig. 1B). Arginase A₄ from kidney contained no groups giving a cross-reaction with the antiserum against arginase A₁ from liver.

The cross-reactivity of different forms of arginase was also examined using the antiserum against kidney arginase A₄. In this case only liver arginase A₁ failed to give a cross-reaction (Fig. 2A, IVb), whereas the remaining four forms reacted with antiserum, giving partial identity arcs (Fig. 2A).

Formation of spurs indicated that some antigenic determinants of arginase A₄ from kidney were absent in arginase A₁ from kidney, A₂ from salivary glands and A₃ from liver (Fig. 2A), however, the latter three forms showed but partial immunological identity (Fig. 2B). Liver arginase A₁ did not contain determinants responsible for cross-reaction with antiserum against kidney arginase A₄.

The comparison of immunological properties of different arginases in the presence of anti-A₁ serum as well as anti-A₄ serum showed that arginase A₁ from kidney, A₂ from salivary gland and A₃ from liver have two types of antigenic determinants.

The two remaining arginases, A₁ from liver and A₄ from kidney are characterized by entirely different antigenic determinants and precipitate exclusively with the specific antiserum (Fig. 1, IVa and b; Fig. 2A, IV a and b). In the double immunodiffusion test in the presence of both antisera, these arginase forms gave two precipitation lines crossing each other, which proved their complete immunological incompatibility (Fig. 3, I a and b).

EDTA-treated preparations retained antigenic determinants characteristic for native forms. Subunits of A₁ from liver precipitated exclusively with anti-A₁ serum while subunits of A₄ only with anti-A₄ serum (not shown). In the presence of both kinds of antisera, the two types of subunits showed complete immunological incompatibility just like the native forms (Fig. 3, II a and b).

Arginase A₁ from kidney, A₂ from salivary glands and A₃ from liver dissociated into subunits and precipitated by both kinds of antisera, also gave crossing precipitation arcs. This confirmed the presence of two antigenic determinants of these arginases and consequently proved the presence of two different types of subunits in each form.

The hybrid nature of arginases has been confirmed by immunoelectrophoresis of EDTA-treated preparations of hybrids. As exemplified by arginase A₂ from salivary glands (Fig. 4b) the EDTA-treated preparation was resolved into two protein bands; one of them precipitated exactly as subunits of A₁ arginase from liver (Fig. 4c) and the other as A₄ from kidney (Fig. 4a). The cathodic protein band precipitated solely with the antiserum against arginase A₁ from liver while the anodic protein band — solely with antiserum against arginase A₄ from kidney. The EDTA-treated preparations of arginase A₁ from kidney and A₃ from liver behaved in the same way (not shown).
In contrast to EDTA-treated arginases, the native forms (e.g. arginase A₂ from salivary glands; Fig. 5d) did not separate upon immunoelectrophoresis into two protein bands, like did their dissociation products (Fig. 5b); it migrated as one protein band precipitating near the start in the presence of both kinds of antiserum. Under the same immunoelectrophoretic conditions native arginases A₄ from kidney (Fig. 5a) and A₁ from liver (Fig. 5c) migrated as single bands to the anode and cathode, respectively. Figure 6 illustrates the immunoelectrophoretic behaviour of all the arginases investigated in the presence of antisera against liver arginase A₁ and kidney A₄. Although in the presence of either antiserum the arginases differed in electrophoretic mobility, each of them exhibited the same mobility in the presence of both anti-A₁ and anti-A₄ antisera. Arginase A₁ from liver (Fig. 6, Ie), made up of cationic subunits and arginase A₄ from kidney (Fig. 6, IIA) built of anionic subunits, exhibited cross-reaction arcs nearest to the cathode and anode, respectively. The natural hybrids, kidney arginase A₁, salivary glands arginase A₂ and liver arginase A₃ formed arcs between the two paternal forms. The electrophoretic mobility of these three forms probably depended on the charge of the component subunits.

DISCUSSION

The behaviour of the arginases isolated from rat tissues in the double immunodiffusion test and on immunoelectrophoresis confirmed the heterogeneity of arginases, postulated by Gasiorewska et al. (1970). The present results point to the occurrence in rat tissues of five forms of the enzyme. There are two immunologically distinct paternal forms, arginase A₁ from liver and A₄ from kidney, made up of identical subunits. Besides, there are three hybrids (as it was suggested earlier by Skrzypek-Osiecka & Poremska, 1983), each built of two types of subunits, showing cross-reaction with the antisera specific for the two paternal forms.

Gasiorewska et al. (1970) regarded arginase A₁ from kidney as identical with A₁ from liver since both enzymes displayed a similar affinity to DEAE-cellulose and the same electrophoretic mobility on polyacrylamide gel. However, the present immunological studies demonstrated that in arginase A₁ from kidney some antigenic determinants of A₁ from liver are absent. Arginase A₁ from kidney showed cross-reaction with both kinds of antisera, while A₁ from liver precipitated only with the specific antiserum. On immunoelectrophoresis, arginase A₁ from kidney migrated towards cathode slower than arginase A₁ from liver. Accordingly, it can be concluded that arginase A₁ from kidney is distinct from liver A₁, and kidney arginase A₁ can be considered as the fifth arginase form present in rat tissues.

The nomenclature of arginases proposed by Gasiorewska et al. (1970) is inconsistent with the later Recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (1972); moreover it takes into consideration only four forms of arginase known at that time, instead of five forms found in more recent experiments (Skrzypek-Osiecka & Poremska, 1983, and the present paper). In the IUPAC-IUB Recommendations published in 1979 this problem has not been discussed. Therefore, we propose to adopt new symbols for the arginases found in rat
tissues: in agreement with the Recommendations of the IUPAC-IUB Commission (1972), the enzyme form with the highest electrophoretic mobility is denoted by index 1. The former nomenclature of arginases (Gąsiorowska et al., 1970) and that proposed for the present current use are presented in Table 1.

Table 1

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<th>Nomenclature of arginases</th>
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REFERENCES


WŁAŚCIWOŚCI IMMUNOLOGICZNE ARGINAZ Z TKANEK SZCZURA

Streszczenie

Na podstawie właściwości immunologicznych wykazano, że w tkankach szczura występuje pięć form arginazy, które zaczynając od formy najbardziej anionowej oznaczono symbolami: A₁ (nerka), A₃ (wątroba), A₅ (gruczoły słynowe), A₄ (nerka), A₅ (wątroba).

Arginazy A₁ i A₃ są formami macierzystymi, zbudowanymi z jednego rodzaju podjednostek. Podjednostki obu arginaz nie wykazują pokrewieństwa immunologicznego. Pozostałe arginazy A₅, A₃ i A₄ są hybrydami obu typów podjednostek.

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