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PURIFICATION AND SOME PROPERTIES OF HUMAN HEART
ARGINASE**

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Arginase from extracts of human heart was purified about 1500-fold. In poly-
acrylamide-gel electrophoresis the enzyme migrated to the cathode at pH 5.5, show-
ing very low mobility at pH 8.9. Molecular weight determined by gel filtration was
120 000. The $K_m$ for L-arginine was 5 mm. L-Ornithine and L-lysine were competitive
inhibitors. The enzyme was completely inactivated by treatment with EDTA, and
dissociated into subunits with mol.wt. of about 30 000. Addition of Mn$^{2+}$ ions to
the inactive subunits resulted in reappearance of the enzyme activity; the molecular
weight of the reactivated enzyme corresponded to that of the native form.

It has been postulated that arginase occurs in human tissues in molecular
forms differing in electric charge and in behaviour on ion-exchange chromato-
graphy (Bascue et al., 1966; Porembeka & Kędra, 1971; Borcio & Strans,
1976). Since it has been demonstrated that determination of arginase
activity in blood serum may serve as a useful test in early differential
diagnosis of myocardial infarction (Porembeka & Kędra, 1975), we have
purified the enzyme from human heart and compared its molecular char-
acteristics with those of the enzyme from other tissues.

EXPERIMENTAL

Reagents. These were from the following sources: L-arginine, D-arginine,
L-homoarginine hydrochloride, β-guanidinopropionic acid, L-α-amino-guan-
dinopropionic acid hydrochloride, γ-guanidinobutyrate, α-amino-β-guanidino-

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butyrate (Calbiochem, Los Angeles, Calif., U.S.A.), acrylamide and methylene-bisacrylamide (Fluka, Buchs, Switzerland), N,N,N',N'-tetramethylethylenediamide (Eastman Organic Chemicals, Rochester, N.Y., U.S.A.), Sephadex G-100 and G-150 and Blue Dextran 2000 (Pharmacia, Uppsala, Sweden), CM-cellulose (CM-52) and DEAE-cellulose (DE-11), (Whatman Biochemicals, Maidstone, Kent, England). Marker proteins: bovine albumin, chicken ovalbumin, bovine globulin and horse myoglobin (Sigma Chem. Co., St. Louis, Mo., U.S.A.). All other chemicals were the purest available grades from standard commercial sources.

Hearts were taken within 20 - 36 after death of persons 20 - 50 years old, killed in traffic accidents, with no pathological changes observed at autopsy.

Arginase assay. Arginase activity was measured by determining the increase in the amount of the reaction products, urea and ornithine. Urea was determined according to Ratner (1955), ornithine by the method of Chinard (1952) as modified by Poremska & Barańczyk-Kuźma (1974). Extinction was measured in a Spectronic 20 photocolorimeter (Bausch-Lomb) at 540 nm for urea and 515 nm for ornithine. One unit of enzyme activity was defined as 1 μmol of product formed/min at 37°C.

Protein determination. The content of protein was determined according to Lowry et al. (1951) with crystalline bovine serum albumin as standard or spectrophotometrically by the method of Warburg & Christian (1941).

Polyacrylamide-gel electrophoresis. Electrophoresis was performed in Tris/glycine buffer, pH 8.9 (Davis, 1964) and in acetate buffer, pH 5.5 (Sakai & Gross, 1967) at 3 mA/tube. Protein was stained with 0.5% Amido Black in 7% (v/v) acetic acid. Arginase activity was detected in 2-mm gel slices eluted with 1 ml portions of barbitone buffer, pH 9.5.

Molecular-weight determination. This was determined by Sephadex G-100 and Sephadex G-150 chromatography, as described by Andrews (1964). Horse myoglobin (mol.wt. 17 000), ovalbumin (mol.wt. 46 000), bovine serum albumin (mol.wt. 69 000), bovine γ-globulin (mol.wt. 150 000), were used as molecular weight standards (5 mg each). Fractions (2 ml) were checked for arginase activity and protein content.

EDTA-treatment of human heart arginase. The enzyme obtained after the 6th purification step (Table 1) was concentrated in the presence of Ficoll and then dialysed against 50 mm-Tris/HCl buffer, pH 7.5. This preparation (2 mg of protein) was incubated with 50 mm-EDTA at pH 7.5 and 37°C for 30 min. Arginase activity was determined in the presence of Mn²⁺ and without Mn²⁺ added. The molecular weight of EDTA-treated arginase was determined by Sephadex G-100 chromatography; the column was previously equilibrated with 10 mm-EDTA.
RESULTS

Purification of human heart arginase. For purification of the enzyme the procedure described by Schimke (1964), Tarrab et al. (1974) and Barańczyk-Kuźma et al. (1976) for rat liver and kidney arginase, was used. Unless otherwise stated, all procedures were carried out at 4°C.

The results of purification are summarized in Table 1. The specific activity of the purified heart enzyme was 1500-fold that of the crude heart extract. The preparation contained 0.8 mg protein. The overall recovery was 51%. The major difficulty encountered in the purification of human heart arginase was low enzyme concentration and its low stability.

Table 1

<table>
<thead>
<tr>
<th>Step</th>
<th>Procedure</th>
<th>Total protein (mg)</th>
<th>Total activity (units)</th>
<th>Specific activity (units/mg)</th>
<th>Purification factor</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Extract</td>
<td>2450</td>
<td>24.5</td>
<td>0.01</td>
<td>—</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>45 - 75% (NH₄)₂SO₄, sat.</td>
<td>492</td>
<td>23.6</td>
<td>0.048</td>
<td>4.8</td>
<td>96</td>
</tr>
<tr>
<td>3</td>
<td>Heating at 60°C, 20 min</td>
<td>180</td>
<td>23.4</td>
<td>0.13</td>
<td>13.0</td>
<td>96</td>
</tr>
<tr>
<td>4</td>
<td>Ethanol precipitation</td>
<td>13.8</td>
<td>22.0</td>
<td>1.6</td>
<td>160.0</td>
<td>89</td>
</tr>
<tr>
<td>5</td>
<td>DEAE-cellulose</td>
<td>3.9</td>
<td>18.0</td>
<td>4.6</td>
<td>460.0</td>
<td>73</td>
</tr>
<tr>
<td>6</td>
<td>CM-cellulose</td>
<td>2.3</td>
<td>16.2</td>
<td>7.2</td>
<td>720.0</td>
<td>66</td>
</tr>
<tr>
<td>7</td>
<td>Sephadex G-150</td>
<td>0.8</td>
<td>15.0</td>
<td>12.5</td>
<td>1500.0</td>
<td>51</td>
</tr>
</tbody>
</table>

Properties of the enzyme. The enzyme after the 7th step of purification exhibited in disc-gel electrophoresis a high degree of purification. At pH 5.5 and 8.9 and at various protein concentrations, only one major band of protein with arginase activity and only one minor protein band contaminating the enzyme were visible (Fig. 1). At pH 5.5, the two distinct protein bands migrated towards the cathode (Fig. 1). The total arginase activity was found in the faster moving fraction. Electrophoresis at pH 8.9 also gave two protein bands, but in this case the inactive protein migrated towards the anode whereas the enzyme exhibited very low electrophoretic mobility and was revealed in the first band near the start.

The heart enzyme was adsorbed on CM-cellulose and emerged as a single symmetrical peak.

Both electrophoretic and chromatographic behaviour indicate that in human heart only one form of arginase, showing cationic property, is present.

The molecular weight of human heart arginase, estimated by gel filtration as described by Andrews (1964) on a Sephadex G-150 column, was 120 000.

EDTA inactivated human arginase, the degree of inactivation being dependent on the amount of EDTA added and the time of treatment. The
Fig. 1. Polyacrylamide-gel electrophoresis of native and EDTA-treated human heart arginase. The final preparation of the enzyme (20 μg of protein) was analysed as described in Methods at: a, pH 5.5; b, pH 8.9; and c, the 30,000 subunits (10 μg of protein) obtained on EDTA-treatment following concentration by Ficoll and dialysis in 0.025 mM-Tris/glycine buffer, pH 8.9.

Preparation completely inactivated by EDTA gave on calibrated Sephadex G-100 and G-150 columns a single fraction with a molecular weight of 30,000; its activity depended on the addition of Mn²⁺. In polyacrylamide-gel electrophoresis at pH 8.9, the 30,000 subunits migrated towards the anode as a single protein band, showing greater mobility than the native enzyme (Fig. 1c).
The behaviour of the human heart arginase subunits (30 000) subjected to electrophoresis indicated that the enzyme is composed of a single type of subunits. The electrophoretic mobility as well as molecular weight of the preparations reactivated by Mn$^{2+}$ were the same as those of the native enzyme.

pH optimum was 9.4 in 100 mM-Tris/HCl buffer, 100 mM-sodium barbitone buffer or 250 mM-glycine/NaOH buffer.

Human heart arginase showed high specificity for the L-isomer of arginine and did not hydrolyse the D-isomer of arginine, D-homoarginine and the other compounds tested. With L-canavanine only trace activity was observed.

From the double-reciprocal plot of the initial rate and substrate concentration, the Michaelis constant of the purified enzyme was found to be 5 mM for L-arginine in glycine/NaOH buffer, pH 9.4, in the presence of 5.0 mM-MnCl$_2$ (Fig. 2).

Fig. 2. Double-reciprocal plot of human heart arginase activity versus L-arginine concentration. Assays were performed as described in the text using 2 µg of the purified enzyme. L-Arginine was used as a substrate for $K_m$ determination. L-Ornithine and L-lysine were added in 10 mM concentration. $v$ is expressed as µmol/(min × mg). (○), Without inhibitor added; (●), lysine; (△), ornithine.

As shown in Table 2, valine, isoleucine and tryptophan at a concentration of 10 mM acted as strong inhibitors of human heart arginase, whereas aspartate, glutamate, glycine, serine and phenylalanine caused but a slight inhibition. The affinity for the inhibitors, L-ornithine and L-lysine (0.04 M) was determined at different concentrations of L-arginine. The results obtained indicate competitive inhibition of the enzyme (Fig. 2).
Table 2

Effect of various L-amino acids on the activity of human heart arginase
Enzyme from step 6 (5 µg of protein) was used and the activity was measured in the presence of the amino acids listed, at a concentration of 10 mM. The inhibition refers to arginase activity determined without inhibitors added.

<table>
<thead>
<tr>
<th>Addition</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate</td>
<td>2</td>
</tr>
<tr>
<td>Glutamate</td>
<td>5</td>
</tr>
<tr>
<td>Glycine</td>
<td>5</td>
</tr>
<tr>
<td>Serine</td>
<td>9</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>9</td>
</tr>
<tr>
<td>Threonine</td>
<td>31</td>
</tr>
<tr>
<td>Histidine</td>
<td>39</td>
</tr>
<tr>
<td>Alanine</td>
<td>51</td>
</tr>
<tr>
<td>Leucine</td>
<td>56</td>
</tr>
<tr>
<td>Ornithine</td>
<td>59</td>
</tr>
<tr>
<td>Proline</td>
<td>61</td>
</tr>
<tr>
<td>Lysine</td>
<td>63</td>
</tr>
<tr>
<td>Methionine</td>
<td>64</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>75</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>84</td>
</tr>
<tr>
<td>Valine</td>
<td>84</td>
</tr>
</tbody>
</table>

DISCUSSION

The presented results demonstrated that arginase in human heart is not polymorphic, resembling in this respect the enzyme from lung (Dahlig et al., 1975), leukaemic lymphocytes and granulocytes (Reyero & Dorner, 1975), and placenta (Porta et al., 1976). In human liver, kidney, erythrocytes and mammary glands, multiple molecular forms of arginase were shown to be present (Borcič & Strans, 1976). Two forms were found in human liver and erythrocytes (A₁, A₄), (Cabello et al., 1966; Bascur et al., 1966) and in human kidney (A₁, A₄), (Poremb ska & Kędra, 1971).

Human heart arginase is a cationic protein, like forms A₁ from liver and kidney (Poremb ska & Kędra, 1971) and the enzyme from lung (Dahlig et al., 1975), leukaemic lymphocytes and granulocytes (Reyero & Dorner, 1975). It differs from the arginases A₃ and A₄ present in human (Poremb ska & Kędra, 1971) and rat liver and kidney (Kaysen & Strecker, 1973) or rat small intestine (Fujimoto et al., 1976), which migrated towards the cathode under the same electrophoretic conditions. The affinity to CM-cellulose of arginase from human heart resembled that of form A₁. Under the same conditions, the liver form A₃ and kidney form A₄ were not retained on CM-cellulose but they were adsorbed, with different affinity, on DEAE-cellulose.
Molecular weight of human heart arginase, 120,000, is similar to that of arginases from human liver (Carvajal et al., 1971), lung (Dahlig et al., 1975), lymphocytes and granulocytes (Reyero & Dorner, 1975), and livers of other mammals (Poremb ska, 1973). Molecular weight of arginase from human placenta (70,000; Porta et al., 1976) and from rat mammary gland (42,000 and 94,000; Glass & Knox, 1973) was distinctly lower.

The pH optimum and substrate specificity of human heart arginase were similar to those for other arginases from mammalian tissues (Poremb ska, 1973).

The affinity of human heart arginase towards L-arginine at pH 9.5 ($K_m$, 5 mM) was somewhat lower than that of the rat liver enzyme (2.4 mM; Schimke, 1964) but considerably higher as compared with arginase from rat kidney ($K_m$, 18 mM; Kaysen & Strecker, 1973), rat intestine ($K_m$, 19 mM; Fujimoto et al., 1976) or human placenta ($K_m$, 27 mM; Porta et al., 1976).

The degree of inhibition of heart arginase by amino acids was very similar to that of the rat kidney and intestine enzymes. However, L-lysine and L-ornithine inhibited competitively heart and intestine arginase but showed a mixed-type inhibition of the rat kidney (Kaysen & Strecker, 1973) or human lymphocyte and granulocyte enzymes (Reyero & Dorner, 1975).

Like most mammalian arginases, the heart enzyme seems to have oligomeric structure. On treatment with EDTA it became completely inactivated and it dissociated into subunits with molecular weight of about 30,000. Addition of Mn$^{2+}$ ion to the subunits resulted in reappearance of the enzymatic activity. The enzyme is composed of a single type of subunits, like the arginase $A_1$ from rat liver and like form $A_4$ from rat kidney where only one type of subunits of 30,000 mol. wt. was resolved by electrophoresis on polyacrylamide gel at pH 8.9 (Barańczyk-Kuźma et al., 1976).

Anionic arginases from rat intestine (Fujimoto et al., 1976) and calf liver (Dahlig & Poremb ska, 1977) were reversibly inactivated on removal of Mn$^{2+}$ by EDTA; they did not dissociate and their molecular weight proved to be unchanged even at high EDTA concentration. This may indicate a monomeric structure of the anionic arginases. However, the cationic arginase from rat kidney (Barańczyk-Kuźma et al., 1976) dissociated into subunits on treatment with EDTA, and anionic ox liver arginase split into subunits at low pH, although it did not dissociate on EDTA treatment (Harrel & Sokolovsky, 1972). This behaviour could be ascribed to different binding of Mn$^{2+}$ by various arginases and to some differences in the structure of enzymes from different sources.

Summing up, the human heart arginase described in this paper seems to be similar to the enzyme from lung, lymphocytes, granulocytes and the form $A_1$ prevailing in liver, but different from the form $A_4$ predominating in kidney and intestine. In view of these data, the distinction between
hepatic and extrahepatic arginases, postulated by Glass & Knox (1973), seems to be insufficiently substantiated.

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OCZYSZCZANIE I NIEKTÓRE WŁAŚCIWOŚCI ARGINAZY Z LUDZKIEGO SERCA

Streszczenie

Arginaza z ekstraktów z serc ludzkich została oczyszczona 1500-krotnie. Podczas elektroforezy w żelu poliakrylamidowym w pH 5,5 enzym wykazujący bardzo niską ruchliwość w pH 8,9 wędrował do katody. Masa cząsteczkowa oznaczona metodą sączenia na żelu wynosiła 120 000. K_m dla L-argininy wynosiła 5 mm; L-ornityna i L-lizyna hamowały kompetencyjnie arginazę z serca człowieka. Działanie EDTA prowadziło do całkowitej inaktywacji enzymu i jego dysocjacji na podjednostki o masie cząsteczkowej około 30 000. Dodanie jonów Mn^{2+} do nieaktywnych podjednostek przywracało aktywność enzymatyczną. Masa cząsteczkowa reaktywowanej arginazy odpowiadała masie natywnego enzymu.

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