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Separation of Arginase Isoenzymes from Human Tissues by Agar Gel Electrophoresis

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Summary: Arginase (EC 3.5.3.1) from human liver, kidney, mammary gland, and erythrocytes, was separated by agar-gel electrophoresis using barbital buffer pH 8.6. Three isoenzymes were separated. Two of these, A_2 and A_3, occur in liver and erythrocytes. The same two isoenzymes were found in the kidney, but in reversed proportions. In addition to the A_3 isoenzyme, the mammary gland contains a fast anodically moving A_1 isoenzyme. The three isoenzymes differ in their degree of sensitivity to ornithine.

Elektrophoretische Trennung der Arginase-Isoenzyme aus Geweben des Menschen durch Agargelelektrophorese

Zusammenfassung: Arginase (EC 3.5.3.1) aus menschlicher Leber, Niere, Brustdrüsen und Erythrocyten wurde elektrophoretisch in Agargel getrennt. Drei Isoenzyme wurden gefunden. Zwei von ihnen, A_2 und A_3, kommen in Leber und Erythrocyten vor. Die selben Isoenzyme befinden sich auch in der Niere, aber in umgekehrtem Verhältnis. Neben dem A_3-Isoenzym befindet sich in der Brustdrüse ein rasch anodisch wanderndes A_1-Isoenzym. Ornithin hemmt die Aktivität der drei Isoenzyme verschieden stark.

Introduction

Differences among arginases (*L*-arginine amidinohydrolase, EC 3.5.3.1) from various tissues were noted a long time ago when it was shown that the conditions for measuring arginase activity in one tissue were not equally suitable for arginase tests in another tissue (1). Differences in the pH activity curves (2) and immunochemical properties were also noted (3). Using electrophoretic (4) and chromatographic techniques two isoenzymes of the mammary gland have been found (4, 5, 6), while the results concerning the number of liver and erythrocyte arginase isoenzymes are not consistent (4, 5, 6, 7, 8). This paper deals with the technique set up in our laboratory for the separation of arginase isoenzymes and with the results obtained with arginase from various human tissues.

Material and Methods

Tissues were obtained within six hours post mortem. They were washed with saline, freed of connective tissue and of fat which is particularly abundant in the mammary gland. Portions of 0.2 g of tissue were homogenized in a *Potter Elvehjem* homogenizer in 1 ml of 5 g/l Triton X-100 in 0.5 mmol/l $MnCl_2$ solution. The homogenates were centrifuged at 14,000 *g* for 20 minutes the supernatants lyophilised and kept dry at + 4°C until tested. Prior to the analysis they were dissolved in water to give solutions equivalent to 0.15 mg of fresh liver tissue, and 2–3 mg of kidney or mammary gland tissue per ml. Hemolysates were prepared by freezing and thawing of erythrocytes which had been separated from plasma and washed three times with saline. Total arginase activity in tissues and hemolysates was determined by the method described by *Jergović* et al (9), but with a shorter incubation time. Activity is expressed as U/g of fresh tissue or, in the case of hemolysates, per gram of hemoglobin. Electrophoresis was performed on microscope slides in 10 g/l Difco Noble agar (10) dissolved in barbital buffer, pH 8.6, ionic strength 0.02 at 7 V/cm, for 120 minutes. The isoenzymes were located in two ways, by ornithine and by the urea formed in the enzyme reaction.

For the localisation of arginase activity in the gel with ornithine we developed a method based on the reaction of ornithine with ninhydrin in acidic medium (11, 12). When electrophoretic run was finished the gel was covered with Whatman No. 1 filter paper, impregnated with a solution of arginine and activator of the same concentrations, as in the determination of total activity. This sandwich was incubated at 37°C for 30 minutes, the paper stripped off, dried, dipped for a moment in a 50 g/l solution of ninhydrin in equals parts of acetone and propanol, left at room temperature until the colour appeared and then sprayed with a 500 g/l solution of trichloracetic acid in acetone. Faint red spots thus obtained indicated the positions of arginase activities.

For the quantitative determination of isoenzymes with urea the gel was cut into 5 mm wide segments and each segment was transferred to a test tube containing 1 ml of distilled water, and arginase activity was determined in the same way as for the total activity. To test the inhibitory effect of *L*-ornithine on the electrophoretically separated isoenzymes, *L*-ornithine was added to the reaction mixtures in the concentration of 77 μmol/l, equimolar with arginine.

Results

The electrophoresis of liver, erythrocyte, kidney and mammary gland arginase yielded three different isoenzymes that we designated A_1, A_2 and A_3. Two of