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Human Erythrocyte Arginase Purification and Characterization

[1]Fayhaa.M.Khaleel[1]Noor N- Oda [2]EssamFadelAlwan Al-Jumaily
[1]*Chemistry Dept. College of Science for Women, University of Baghdad, Baghdad, Iraq*
[2]*Genetic Engineering and Biotechnology Institute, University of Baghdad, Baghdad, Iraq*
Corresponding Author: Fayhaa.M.Khaleel

Abstract: *Arginase was purifiedfrom human erythrocytes. The purification steps included acetone precipitation, DEAE-cellulosechromatography and gel filtration on Sepharoe6B.The molecular weight of native arginase was find to be (94.406Kd), by gel filtration.The kinetic properties determinedfor the purified human erythrocytesarginaseshowed an optimum pH of 10 and anoptimumtemperature 45 °C. The Km for L-argininemonochlorideand V_{max}values forhuman erythrocyte arginase found to be (200mM), and (47(Unit/ml)$^{-1}$) respectively*

Keywords: *Purification, Human erythrocytes arginase, Kinetic properties*

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I. Introduction

L-Arginase (L-arginine amidino hydrolase, EC 3.5.3.1.) is hydrolase and a metalloenzyme that response to hydrolysis of L-arginine to result urea and L-ornithine (1). There are atleast two forms of arginase. Arginase cytosolic and is most abundant in the liver plays an important role in the ammonia detoxification in mammals, second isoenzyme, arginase II in extrahepatic mammalian tissueskidney, brain, intestine, mammary gland, erythrocytes, and skin(2,3), is involved in the production of ornithine as a precursor to proline, glutamate orpolyamines,such as spermine and putresine, essential for cellular growth(4)

Increase the activity of arginase is associated with various diseases by reducing the supply of L-arginine needed by NOS to produce nitric oxide(plays an important role in homeostatic vasodilatation, regulation of blood flow (5), and alsoit is thought that it plays an important role in cancer growth as a preventative and therapeutic agent(6)), and by raising production of L-ornithine resulting in vascular structural problems (7) and development of carcinogenesis (8).Since this enzyme is important so it is important to study its properties which were not fully studied for that purified from erythrocyte**.**

II. Experimental

Materials and methods

The chemical reagents that are used: Sodium chloride,(Fluka ,Switzerland), aceton, phosphate buffer,Coomassie Brilliant Blue,Bovine serum albumin,Acetic acid(BDH**,** England)**,** Sepharose 6B, DEAE- Cellulose,(Pharmacia Fine Chemicals,Sweden),Tris-HCl, Blue dextran, (Sigma, USA),

Arginase assay

Arginase activity during purification was measured as Zofia and Maria (9) described. Briefly the reaction was done in asystem containing 0.1 M Sodium barbitone(pH 9.5), 200 mM Substrate solution L-arginine monochloride (PH 7.5), 50 mM Manganese chloride solution, and the sample in a final volume of 1.2 ml;the incubationwas carried out for 30min at 37°C. The reaction was stopped with Trichloroacetic acid 20%, The ornithine concentration formed was determined by ninhydrin reaction (ninhydrin reagent and concentrated acetic acid added to the incubation medium), carried out in a boiling water bath for 60

minutes. Then the colored product was determined spectrophotometerically at 515 nm.Ornithine concentration was calculated from calibration standard curve, and the arginase activity expressed as µg of ornithine/min per 1000 ml.
Soluble protein was determinedbythe Bradford method (10).

Purification ofarginase from human erythrocytes
The purification steps oferythrocyte arginase were adapted from Joseph et.al (11).Human blood was obtained from blood-bank of Al-Khalis hospital-Iraq with blood type of O+. The blood was washedwith 0.9 % NaCl,and centrifuged off at (6000 rpm) for 10 min under -4^{0}C.The precipitate washed with the phosphate buffer (PH 7) twice and centrifuged off at (6000 rpm) for 10 min and the supernatant was kept as crude where the purification steps applied on.

The supernatant obtained above was precipitated by acetone where, cold acetone was mixed with the crude enzyme in a ratio (1:1) (v/v) in ice bath, the mixture is centrifuged at 9000 rpm for 10 min, the precipitate was dissolved in a small amount of 0.2M phosphate buffer pH 7 and dialyzed against the same buffer, then volume, an enzyme activity and protein concentration was measured.

DEAE-Cellulose Ion Exchange Chromatography:

Treated DEAE-Cellulose was packed in a columnof (3×10 cm) andequilibration was done with(pH 7.4) buffer of 5 mMTris-HCl.The dialyzed protein from the preceding step was then layered on the column. The column was first washedwith0.1 M Tris-HCl buffer, pH 7.4, followed by NaClstepwise gradient ranging (0.05-0.5M) prepared in 5 mMTris-HClbufferis used to elute the bounded proteins, from the resin. The fractions of 5ml were collected, performed at a flow rate 1ml/min. Protein wasmeasuredspectrophotometrically at280 nm, the fractions were also assayed for arginase activity.The active fractionswere pooled together.

Gel Filtration Chromatography

Column of (1.5×65cm) waspacked with Sepharose 6B Gel filtration, column equilibrated with Tris-HCl buffer (0.1M, pH 7.4). Above pooled fractions wereconcentrated andapplied on the Column.5ml of fractions were collected at 1ml/min. Protein was measured spectrophotometrically at 280 nm and for arginaseactivity wasassayed.The active fractionswere pooled together

The native molecular weight was determined by gel filtration on Sepharose 6B. The standard proteins were*Casein (*31Kd**),** LPS for *Klesiellapneumoniae*(52.480 Kd), Bovine serium albumin (67 Kd), Superoxidasedismatase (89 Kd), Catalase (232 Kd).

Optimum pH
The pH dependence of the enzymatic activity ofarginase from human erythrocytes was determined byusing two buffer systems: sodium phosphate (pH 8.0 to 9.0) and barbiton buffer(pH 9.5 to 11.0).

Effect of temperature
Various temperature values ranged (30-70) °C are used to determine the preincubationeffectoftemperature on enzyme activity. The activity was determined after the incubation of the purified enzyme for one hour. at different temperature (30, 35, 40, 45, 50, 55, 60, 65, and 70) °C.

Kinetic Parameters:
The kinetic parameters of arginase (V_{max} and K_m) were determined by using various concentrations of the substrate L-arginine monochloridesolution (100,150,200,250,300mM). The kinetic parameters were determined from the double reciprocal plot Lineweaver-Burk.

III. Results And Discussion

Enzyme Purification

Table (1)shows the purification results of human erythrocyte arginase.The specific activity ofthe purified human erythrocyte enzyme (51.85units/mg) was 2.52-fold that of the crude erythrocyte extract.

The treatment with acetone resulted in the increase of specific activity of the enzyme that also reported by Masaki*et.al*. (12), and Joseph et.al.(13).Erythrocytearginasewas not retained by DEAE-cellulose, that agree with finding of George et.al.(14) That the kidney arginasewas not retained by either DEAE- orCM-cellulose, and also agree withOkonji et al(15) that reported the Tortoise (KinixysErosa) Liver Arginase get in washing onDEAE-cellulose ,but it disagree with Joseph et.al.(13)finding that the Erythrocytearginaseapplied to a DEAE-cellulose column and eluted with a linear gradient of KCI. And this may be due to that the enzyme charge is weak and because of the high concentration of the buffer used (0.1M), too salty, that compete with enzyme to bind the resin; the enzyme is not retained by the resin.However,the specific activity in this step was increasedto 34.92units/mg and the enzyme was purified as 1.7-fold that of the crude erythrocyte extract so this step is success to riddance from some of proteins that get on the resin.Applying the enzyme finally to the sepharose6-B gel filtration resulted in increase of specific activity and the fold of purifying with yield of 10.46.

Table (1): The purification of arginase from human erythrocyte

steps	Volume(ml)	Enzyme activity (units/ml)	Protein conc. (gm/ml)	Specific activity (unit/mg)	Total activity (units)	Fold	Yield (%)
crude	80	25.10	1.22	20.57	2008	1	100
acetone	45	28.00	0.98	28.57	1260	1.39	62.74
Ion exchange DEAE-cellulose	20	22	0.63	34.92	440	1.70	21.91
Gel filtration sepharose-6B	15	14	0.27	51.82	210	2.52	10.46

Molecular weight

The molecular weight for human erythrocyte arginase,estimated by gel filtration to give a result of (94.406Kd), and this result approaches to those recorded by Masaki et al (12)who found that arginasemolecular weight to be (105 Kd) in human erythrocyte, Joseph Beruter et.al.(13) (107 Kd)for human liver arginase, Saleh A.et al (16) finding was(92Kd), and Shivraj et.al.(17) reported was (118Kd) for of buffalo liver arginase.

Optimum pH

The Effect of pH on the purified enzyme by using various buffers with pH rang of (8-11) shows that the activity of arginase increased with increasing pH until reach its maximum activity at pH 10 and, then the activity declined at higher pH Fig(4).this result in agreement with those reported with Snehal et al.(18), Nobuoet al.(19) who found the optimum pH of arginasepurified from *Vignacatjang*cotyledon an,*Bacillus subtilis*respectively to be 10.0.

Those Saleh et al. (16) and Okonji et al.(16)found that the optimum pH of Fasciolagigantic Arginase, and Tortoise (KinixysErosa) Liver Arginase respectively to be 9.5, while R. E. Okonji et al.(20) reported that the optimum pH of 8 for Arginasepurified from gut of grasshopper. in general these finding indicate that Arginase appears to showa basic optimumpH of 9.5–10.5, The variance in activitywith pH proposesthat atthe catalytic site an ionisable group may function (18).

Effect of Temperature on Enzyme Activity

Asit noticed in Fig.(5) that the preicubation of the purified enzyme in the range of temperature (40-70) for 1hr. detects the optimum temperature for arginase at 45 °C that approaches to those recorded by Saleh.*et al*(16), Shivraj*et al* (17),R. E. OKONJI et al(21), and M. Ferit (21) who found that the optimum temperature forarginase to be 40°C.Okonji*et al*(15) reported that arginaseoptimum temperature was 60°C.

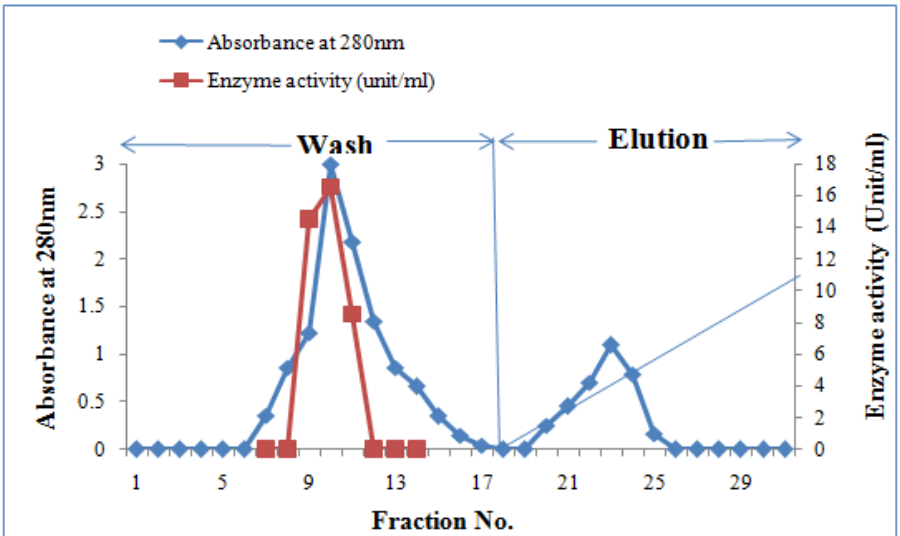

Figure (1): Ion Exhange chromatgraphy for human erythrocyte arginase through DEAE-Cellulose (3.0 x 10 cm) . The column was calibrated with 5mM Tris -HCl pH 7.4 buffer . flow rate 60ml/hrs and 5 ml fraction.

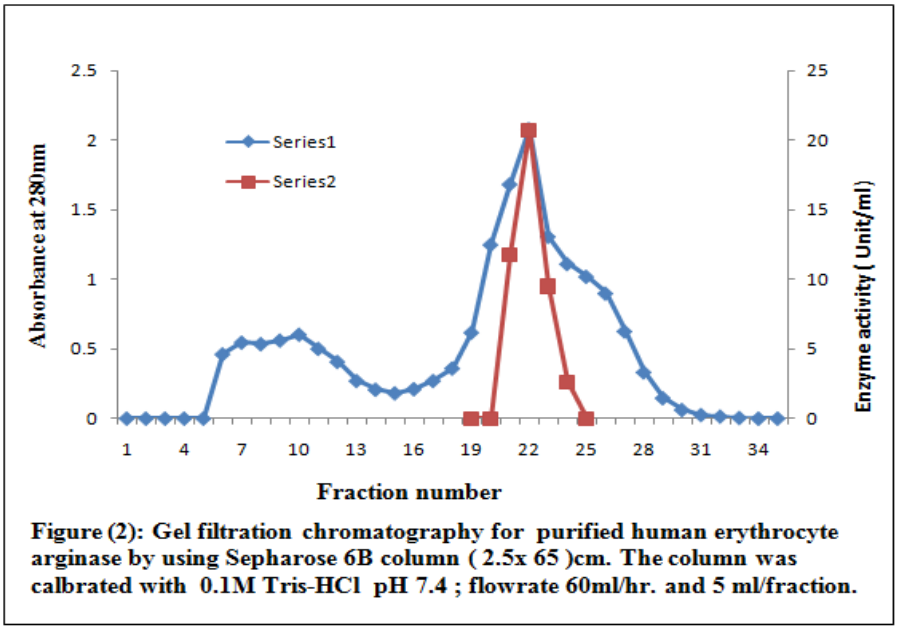

Figure (2): Gel filtration chromatography for purified human erythrocyte arginase by using Sepharose 6B column (2.5x 65)cm. The column was calbrated with 0.1M Tris-HCl pH 7.4 ; flowrate 60ml/hr. and 5 ml/fraction.

Kinetic parameters

The substrate concentration effect on the activityof human erythrocytesarginase was ClarifiedbyLineweaver-Burk plot Fig(6), the relation between the reciprocal of substrate concentration and the reciprocalof velocity, the Km andVmaxvalues forhuman erythrocyte arginase found to be (200mM), and (47(Unit/ml)$^{-1}$) respectively. It can be noticed that the increasing in substrateconcentration enhances the rat of the reaction until the further increasing in substrate

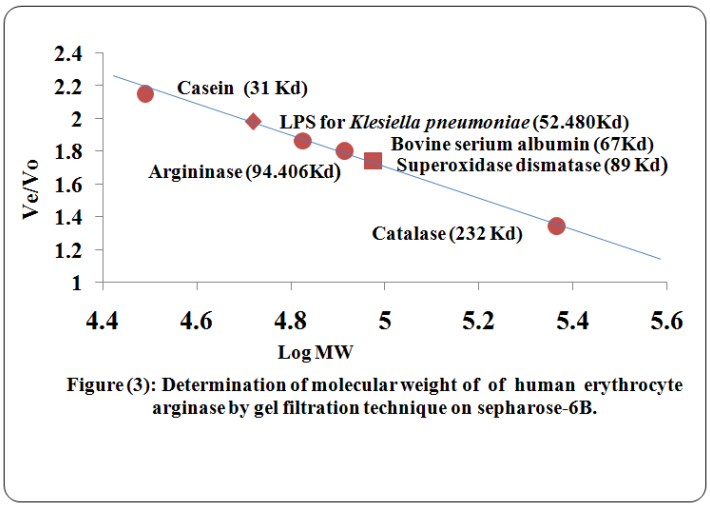

Figure (3): Determination of molecular weight of of human erythrocyte arginase by gel filtration technique on sepharose-6B.

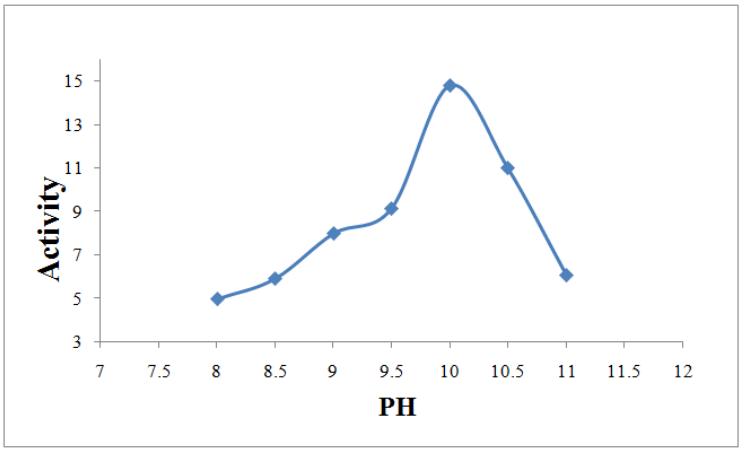

Figure (4): Effect of pH on the human erythrocyte arginase

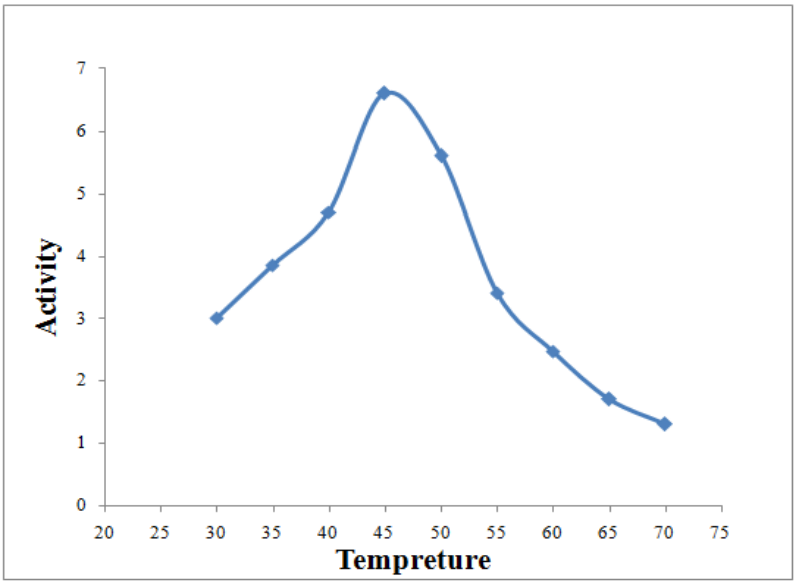

Figure (5): the effect of temperature on human erythrocyte arginase activity

Concentration become without effect on the reaction rat, because the enzyme becomes saturated, and the active site is occupied with the substrate. Ferit, (21) stated that Km value for arginase purified from human Vitreous Humor were 6 mMto L-arginine, R. E. OKONJIet al (21), and Okonjiet al(15) reported thatKm value to L-arginine was (40mM) for arginase purified from gut of grasshopper, and (66.7mM) for arginase purified from liverof tortoise, Ezima et al found that the Km to be (17mM) for

arginine and Vmax value to be (1.39µmole ml-1 min-) for arginase purified from fruit bat liver.Thevariancein millimolar concentration of arginine ismay be belong to the different methods that used and nonphysiologicalconditions generally used (1).

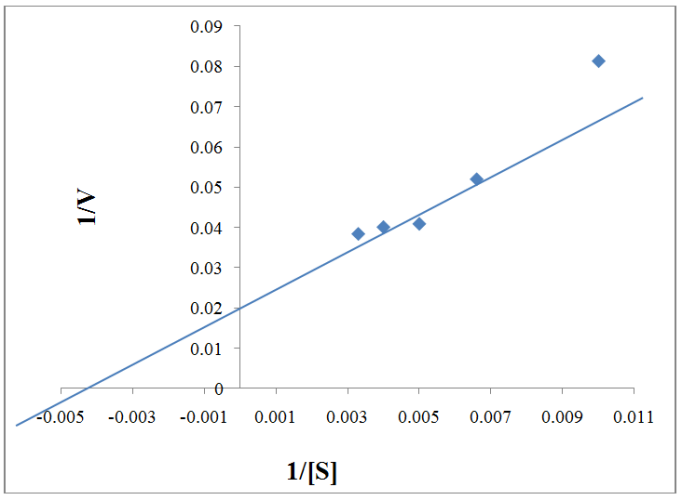

Figure (6): Kinetic constants of arginase purified from human erythrocytes according to Linweaver-Burk

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