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Purification of Arginase from Goat Liver Tissue

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Abstract

The study aimed to purification of Arginase from goat liver tissue, this study included extraction enzyme from local goat liver tissue. The purification process was done with several steps included homogenized crude extract, heat treatment, precipitation with inorganic salt $(NH4)_2SO4$ 30%-70%, ion exchange chromatography by DEAE Sepharose anion column, and size exclusion chromatography by Sepharose 6B. Consequences have depicted a precipitate and concentrated protein with six peaks in ion exchange column. arginase positioned in the first and fourth peak with purification fold (3.8, 3.2) , yield (3.2 ,3.4)) of enzyme and specific activity (99.18 ,82.5)) IU/ml respectively, which obtained a single peak by gel filtration chromatography from isoenzyme I, the degree of purification (8.06) fold, yield of enzyme (4.7) with specific activity (210.7) IU/ml, also, the peak that has the highest enzymatic activity showed single peak after elution in gel filtration chromatography following steps based on SDS- PAGE Electrophoresis .From this paper, it is concluded that arginase purified from goat liver tissue has two isoenzymes with molar mass about 48,000 (±1000) Daltons. Also, it has concluded that purity and molar mass of purify arginase have shown approximately ~ 46 KD with single band by gel filtration chromatography.

Keywords: goat liver tissue, arginase, purification.

Introduction:

The liver is converted to a capsule of the so-called Glisson Capsule, the thin form of connecting tissue filled with collagen fiber. The hilum region thickens this capsule (the position in which a portal vein and hepatic artery go in a liver and left & right hepatic ducts and lymphatics leave the liver). The vessels and ducts have enclosed in the entire origins and ends with connective tissue [(1)]. Each liver lobule consists of millions of liver cells known to be (hepatocytes) the essential metabolic cells, the functional elements in the liver. [(2).]

Kossel and Dakin discovered Arginase, which hydrolyte arginine, in the mammalian liver in 1904 [(3)]. The hydrolysis of L-arginine is catalyzed by a binuclear manganese metalloenzyme which is urea- and ornithine. The enzyme consists of two isoforms, Arginase I besides II. Arginase I stand for the cytosol trimeric protein with a molar mass about 34,700 Da and can be expressed in human and higher primates in erythrocytes. Arginase II stands for as well a mitochondrial trimeric protein with molar mass about 36,100 Da expressing in extrahepatic tissue such as the small intestine, kidney, brain, macrophages and monocytes [(4)] Arginase II has synthesized as a preprotein and imported into mitochondria [5)]

Arginase, which is mainly found in liver, kidney and erythrocytes, converts L-arginine to urea and ornithine, reducing the availability of NOS substrates in order for NO• to be manufactured in these organs [(6)].

Arginase isoforms in humans and are both increasing in inflammatory conditions such as pulmonary hypertension and sickle cell disease [(7,8)] In the early 1930s, the

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identifying of the Krebs – Hensel urea cycle emphasized the importance of arginase; previously known were the individual hydrolytic functions of Arginase, but scientific interests were triggered by an Arginase interdependence and other biochemical mechanisms, which were demonstrated in the urea cycle. Although initial findings have shown that arginase occurs mostly in mammalian liver[9] and less in kidneys [10] this enzyme was as well found in organs that do not have a urea cycle [11-13]

The co-expression of 2 arginase isoforms with a number of induction and feedback loops makes a remarkably complicated signaling cascade for the L-arginine metabolic path. It is crucial to maintain a organism functionality that all these signals are homosexual. Arginase can regulate the development of NO•, polyamines and proline, particularly in immune cells [14], endothelial cells [15], and neuronal cells [16].

The study aimed to purified Arginase from goat liver tissue and determined molar mass and purity of purified enzyme by SDS- PAGE Electrophoresis.

2-Materials and methods:

Extraction of Arginase from Goat liver tissue

Liver sample had been weighed with (30,15,5) gm normalized with 120 ml of chilled Tris-HCl buffer (100 mM, pH 7.5) having 5 mM $MnCl_2$ and 100 mM KCl, 20 Mm L-arginine. A homogenate had been centrifuged under 30 minutes, 10000 rpm, and the supernatant was collected.

Arginase Purification:

Homogenized

Liver sample has weighed with (5 gm) homogenized with 120 ml of chilled Tris-HCl buffer (100 mM, pH 7.5) having 5 mM $MnCl_2$ and 100 mM KCl ,20 mM L- arginine.

Heat treatment

The crude extract that was obtained in the last step of goat's liver in order to enhance coagulation of thermolabile proteins was therapied and supernatant was further removed in order to test their Enzymatic activity. Arginase was found to be stable at 60 ^{o} C from goat liver [17]

Precipitation by using Ammonium Sulfate (NH4) 2 SO4 concentration 30%-70% [18]

The most commonly used method for precipitation of protein is the addition of inorganic salts such as ammonium sulfate or potassium phosphate, to 50 ml of the heat treatment supernatant added gradually amount of ammonium sulfate in a beaker under stirring and cooling condition (4 C^{o}) after this solution became turbid and precipitated , it was separated by using centrifuge at (4000 rpm) for 60 min , the precipitated was dissolved in tris -HCl buffer (50mM , pH=7.5), protein concentration and enzyme activity were determined for each step

Ion Exchange Chromatography

Anionic exchanger column was prepared from DEAE-Sepharose in dimension (14 ×1.5) cm , which washed and equilibrated with tris -HCl buffer (50mM , pH=7.5). The aliquot for the 3ml fraction had collected in every tube with flow rate of 60 ml/hour. An absorbance had been measured under 280 nm for each fraction and fractions for giving the maximum absorbance was collected. Protein concentration and arginase activity for these fractions was determined, and then bound proteins were eluted with 100 ml of the tris -HCl buffer (50 mM , pH=7.5), having linear gradient of 0.1–1.0 M NaCl. The aliquot of the 3ml fraction had collected in every tube under flow rate of 1 ml/min. An absorbance for every fraction has measured at 280 nm by means of UV-VIS spectrophotometer, fractions that provided an

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uppermost absorbance had collected. Enzyme and protein activities had valued for these fractions.

***Gel filtration Chromatography*:**

A sample gotten from ion exchange chromatography has applied to the top size exclusion gel column (Sepharose 6B) column (21 x 1.5 cm) that equilibrated via tris -HCl buffer (50 mM, pH=7.5), then eluted with wash buffer, aliquot of 3ml fractions were collected in each tube with a flow rate of 60 ml / hour, the optical density for fractions were measured at 280 nm through UV-VIS spectrophotometer, fractions that provided a maximum absorbance were collected. Enzyme and protein activities had valued for these fractions.

***Arginase activity assay* [19]**

Single unit of arginase stands for a quantity of enzyme that will transform 1.0 μmole of L-arginine to ornithine and urea for each minute at pH 9.5 and 37 °C..

***Estimation of protein concentration* [20,21]**

Determination of total protein concentration in serum and each purification step was performed by a kit using biuret method provided from a (SPINREACT) company.

***SDS- PAGE Electrophoresis* [22,23]**

A purity and molar mass of Arginase that purified from goat liver tissue have done using SDS-PAGE gel electrophoresis.

Results & Discussion

Purification of Arginase from liver tissue

Crude enzyme that extract from (5 gm) goat liver tissue that homogenized with 120 ml of chilled Tris-HCl buffer (100 mM, pH 7.5) having 5 mM $MnCl_2$ and 100 mM KCl ,20 mM L- arginine.

Heat treatment had applied for a crude extract which gotten from previous step of goat liver for enhancing a coagulation of thermolabile proteins. They were additionally removed through centrifugation, and supernatant has been collected for testing an enzymatic activity. The arginase from goat liver was stable under 60 º C. Like this thermal stability of arginase has as well been testified from livers of beef [24] , rabbit [25], and buffalo [17]

50 ml of crude extract which obtained in the heat treatment step was precipitated with ammonium sulfate (30%-70%), protein precipitated by salting out method which includes added inorganic salt such as ammonium sulfate because it is easily soluble in water, solubility of protein decreased and precipitated owing to the protein charge equivalence by act of NH4(SO4)2 . when salt is added to crude solution, hydrophobic interaction between water and protein occurs as a result of high surface tension. The protein interacts with station through reducing its surface area for reducing contact with a solvent—as manifested by folding (a folded conformation has been more compact as compared to unfolded one) and then self-association leading to precipitation [26]

Ion exchange and gel filtration chromatography have been applied correspondingly in purification of Arginase. Ion exchange chromatography applied by DEAE- Sepharose column (the anionic ion exchanger) which have numerous uses led to its great capacity for bio separation, straightforward preparation and easiness for separating biomolecules [27]

Results in figure (1) showed six protein peaks appeared at wash, (0.1,0.2, 0.3, 0.4, 0.5) M NaCl in 50 mM tris-HCl buffer (pH= 7.5), arginase that purified from goat liver tissue located at 0.1M NaCl in fractions (20- 27), and 0.4 M NaCl in fractions (63-71).

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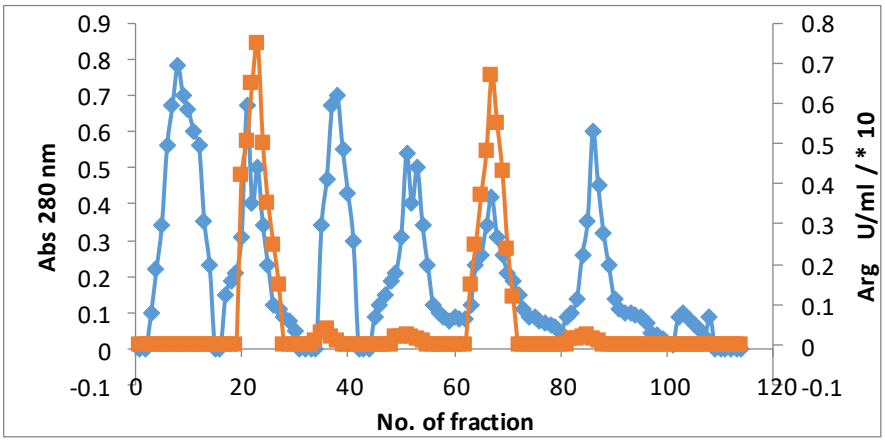

Figure (1): Purification of Arginase from goat liver tissue by Ion Exchange Chromatography

The highest active fractions obtained from ion exchange chromatography which eluted with (0.1 M NaCl) have been pooled and concentrated for applicable gel filtration chromatography via sepharose 6B column.

Results displayed in figure (2) showed a single protein peak after eluted with 50 mM Tris-HCl buffer at pH = 7.5, active Arginase peak appeared in Fraction No. (4-14),

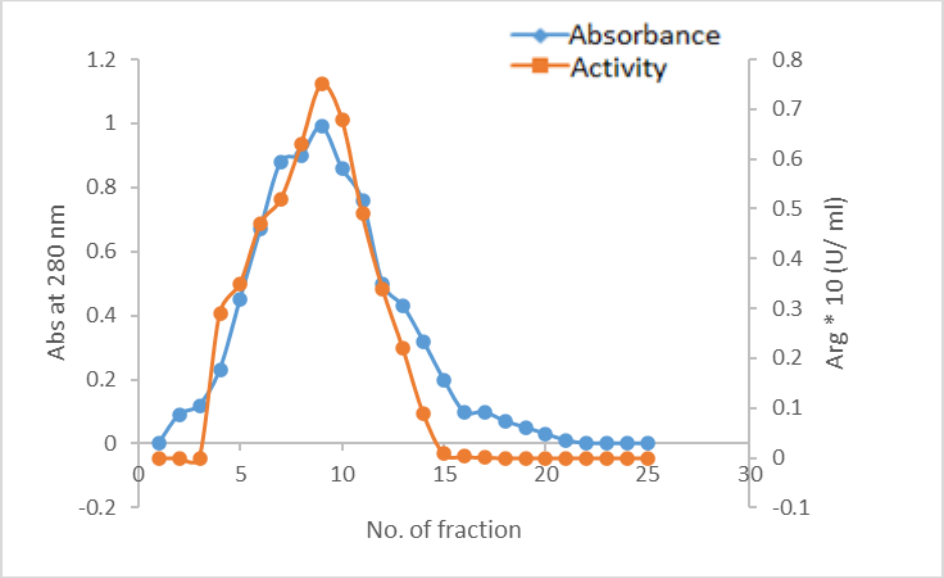

Figure (2) :- Purification of Arginase from goat liver tissue by Size exclusion chromatography

Volume, protein concentration, Arginase activity, particular activity, purification fold and yield for each purification step of Arginase from liver tissue are shown in table (1)

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Table (1): Purification steps of Arginase from goat liver tissue

SDS- PAGE Electrophoresis

Steps	Volume (ml)	Total protein Conc. (mg/ml)	Arginase activity (U/ml)	Arginase total activity U/ml	Arginase Specific Activity (IU/mg)	Purification Fold	Yield (%)
Homogenate	120	3.06	80	9600	26.14	1	100
Homogenate supernatant	100	2.4	77	8700	32.08	1.23	90.6
Heat treatment	90	1.206	70	6300	58.04	2.22	65.6
Participation (NH_4SO_4)	50	1.08	74	3700	62.7	2.4	38.54
Ion exchange Isoenzyme IIII	5	0.8	66	330	82.5	3.2	3.4
Ion exchange Isoenzyme I	5	0.61	60.5	302,5	99.18	3.8	3.2
Gel filtration Isoenzyme I	6	0.356	75	450	210.7	8.06	4.7

Determining Purity and Molecular Weight of Arginase by SDS-PAGE:

Purity and molecular weight of Arginase has been determined through sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) from goat liver tissue

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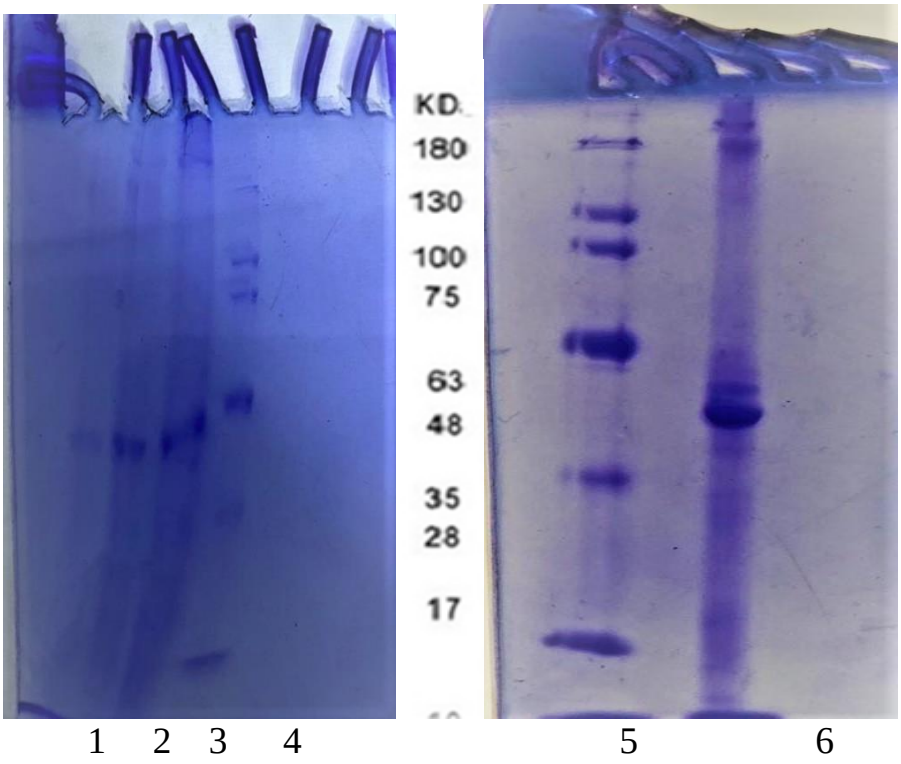

Figure (3): Polyacrylamide gel electrophoresis of purified Arginase.)
lane1: Goat liver arginase after gel filtration by Sepharose 6 B. 2: ion exchange by DEAE-Sepharose 3: crude 4,5: standard 6: Heat treatment supernatant

As shown in figure Arginase that purified from goat liver tissue showed the presence of two narrow bands of two isoenzyme with molar mass about 48,000 (±1000) Daltons by ion exchange chromatography ,and one band with molar mass about ~ 46 KD by gel filtration chromatography for purified enzyme

If it is possible to do extraction and purification the arginase and its isoenzymes from goat liver tissue at several stages. At the early stage, the liver tissue homogenized with buffer containing manganese chloride as co factor and the precipitate were removed by centrifugation and filter paper , degree of purity was obtained at this step (1.23) fold with specific activity (32.08 IU/mg) and (90.6) yield after the homogenization step the crude extraction was thermally treated at 60 ^{o} C .

Heat treatment has applied to a crude extract of goat liver for enhancing a coagulation of thermolabile proteins, that further removed through centrifugation, and supernatant has been collected for testing an enzymatic activity. The arginase from goat liver has been stable at 60 ^{o} C with degree of purity (2.22) and specific activity (58.04 IU/mg) and (65.6) yield. Such thermal stability of arginase has as well been testified from livers of beef [24] and rabbit [25]

Proteins are typically concentrated in primary clearing stages of enzymes by releasing high water and salts, which at some degree can achieve pureness. It is often the protein concentration that occurs with ammonium sulfate since it straightforwardly dissolves in water as salts sedimentation due to protein charge equivalence based on salt act that would cause low protein dissolution along with its sedimentation. This is known as salting out. [28]

The proteins deposited by using ammonium sulfate salt in concentration of (70%) for the concentration of enzyme and purified, 70% arginase saturation with solid ammonium

sulphate has caused an augmented specific activity of liver arginase, degree of purity was obtained at this step (2.4) fold with specific activity (62.7 IU/mg) and (38.54) yield .

As the purification of chromatography by ion exchange is among the significant approaches to separate and purify the enzyme which is based on the principle of the charge difference so the enzyme was purified by using DEAE-Sepharose with using Tris-HCl buffer (50 mM, pH=7.5) containing gradient concentration of sodium chloride, as two isoenzymes were obtained as illustrated in figure (1) and variant purity degree as the degree of purification of I (3.8) fold and (3.2) fold for the isoenzymes IIII

final step the highest activity isoenzyme (isoenzyme I) which obtain from pervious step was purified by gel filtration by using (Sepharose 6B) as the degree of arginase purity at this step reaches (8.06) fold with specific activity (210.7 IU/mg) and (4.7) yield , as illustrated in the table (1).

When using the separation technique gel electrophoresis SDS-PAGA a concentration of 10% using a dye commassie brilliant blue R-250, two isoenzymes were estimated by ion exchange with DEAE-Sepharose and one band by gel filtration

The literatures indicate that two isoenzymes were separated from adult human s liver using CM-cellulose chromatography by (Bascur et al., 1966) [(29)] and also obtain two isoenzymes from rat's liver by(Gasiorowska et al.,1970)[(30)] by using DEAE-cellulose , while (Tarrab et al., 1974)[(31)] three arginase isoenzymes were separated from the rat s liver using CM-cellulose chromatography

Also, arginase from buffalo liver tissue was purified and characterized by DEAE-cellulose chromatography , but no isoenzyme were obtained . An enzyme was eluted in a distinct symmetrical peak, with linear gradient of sodium chloride. The above active enzyme fractions have been passed through Bio-Gel P-150 chromatography and a one homogenous peak of arginase activity has been appeared. We have gotten about 2028.97fold purification from arginine AH-Sepharose 4B affinity adsorbent .[(17)]

From this paper, it is concluded that arginase purified from goat liver tissue has two isoenzymes. Also, it has concluded that purity and molar mass of purify arginase have shown approximately ~ 46 KD with single band.

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