Study The Characteristics Of Partially Purified Arginase From The Serum Of Patients With Diabetes Mellitus Type Two (Dmt2) In The Nineveh Governorate

Ruaa Waleed Alramadhany, Hamodat, Zahraa Mohammed Ali*
Department of Chemistry, College of Science, University of Mosul, Mosul, Iraq

*Corresponding author E-mail:zahraahamodat@uomosul.edu.iq

Doi:10.29072/basjs.20220104

ARTICLE INFO

Keywords

Arginase, Activity, Chromatography, Ion exchange, Gel filtration, Diabetes mellitus type two.

ABSTRACT

Arginase plays an essential role in diabetes mellitus type 2 (DMT2). The study aimed to isolate and partially purify the serum arginase from patients with diabetes mellitus type 2, estimate its molecular weight, and determine the optimal conditions for the enzyme's action. The study included 35 patients of both sexes of 16 males and 19 females. Three purification steps were used to purify arginase are the precipitation of the protein with ammonium sulfate at a saturation of 65%, DEAE-cellulose ion-exchange chromatography, and Sephadex G-100 gel scattering technique. The results showed that the specific activity of unpurified arginine is equal to 0.6 and the specific activity increased 19 times when purified while the specific activity was equal to 11.4 (U/mg). The number of purification times was 19-fold, and recovery of 59.2 %. And the molecular weight of purified arginase equals 96050 ± 1414.2 Daltons. The highest activity of purified arginase in 250 µL serum as a source of enzyme,100 mM of buffer solution sodium barbitone, pH = 9.5, and time incubation for 45 minutes at 37 C, and 200 mM of arginine as a substrate for arginase. The study concluded that the specific activity of arginase from serum of diabetic patients increased 19 times after purification. The properties of arginine and the optimal conditions for its action are differ according to the source of the enzyme purified that from it.

Received 14 Dec 2021; Received in revised form 10 Mar 2022; Accepted 3 Apr 2022, Published 30 Apr 2022

1. Introduction

Arginase (EC 3.5.3.1) is one of the essential enzymes involved in the metabolism and formation of urea[1,2]. It catalyzes Larginine hydrolysis into L-ornithine and urea. Arginase is a part of the urea cycle that regulates L-arginine levels[3,4]. Arginase has two isozymes, firstly in the cytosol in the liver, which plays a significant role in the urea cycle, and the second is located in the mitochondria of the kidneys and prostate [5,6]. Besides, a study has found that patients with diabetes mellitus type two have increased arginase activity [3,7]. In other studies conducted on rats exposed to diabetes, they increased the arginase activity. It was also found that patients with DMT2 have a failure in liver function [3,8,9]. Diabetes mellitus is one of the common diseases that lead to death in the absence of follow-up. It was found that diabetes is on the increase. In addition, according to a study published in 2017, type 2 diabetes affected approximately 462 million people worldwide, accounting for 6.28 % of the global population (4.4 % of those aged 15-49 years, 15 % of those old 50-69 years, and 22 % of those over the age of 70 years) [10], with the number expected to rise to 522 million by 2030 [11]. Diabetes mellitus refers to a group of metabolic disorders in the metabolism of carbohydrates, fats, and proteins, characterized by persistent high blood sugar, which results from a defect in insulin secretion or insulin action. Type 2 diabetes is caused by a defect in the functions of beta cells and their ability to secrete insulin in sufficient quantity [12]. Diabetes mellitus type 2 is present in a range (85-95%) of the total number of diabetics. People with diabetes are more likely to develop heart disease, renal disease, neuropathy, retinopathy, and stroke than non-diabetics. Few people realize that excessive blood sugar levels cause liver illness and cancer[13]. Arginase activity in diabetes patients is higher than in healthy controls [3,13]. Arginase was purified using different techniques from many sources, such as humans and bacteria [14-17]. Our study aimed to isolate and partially purify the serum arginase from patients with diabetes mellitus type 2, estimate its molecular weight, and determine the optimal conditions for the enzyme's action.

2. Materials and methods

2.1 Population study

The study was conducted on 35 people with diabetes mellitus type 2 of both sexes (16 males and 19 females), and their ages ranged from (35-75) years. They were attending Al-Wafa Center and medical outpatient clinics licensed by Nineveh Health. This study was conducted from 1 Sept 2020 to 1 Dec 2021. Moreover, patients with heart, cancer, kidneys and other diseases were excluded. The Ethics Committee of the University of Mosul (College of Science) and the Nineveh Health Department followed the study protocol. All participants were provided with an informed consent questionnaire form to participate in the research.

2.2 Samples collection

Serum samples were obtained by withdrawing 5 ml of venous blood, then placing it in a gel tube and leaving it for 15 minutes at a temp1erature of 37° C and centrifuging 3000xg, then separating the serum and keeping it at a temperature of -20 Ċ until the necessary tests are performed [18].

2.3 Variables Assay

2.3.1 Estimation of arginase activity

The activity of arginase in serum was measured. Based on Kocna et al., 1996 colourimetric and quantitative method. Ornithine is produced by the decomposition of arginine by arginase at a wavelength of 515 nm. The amount of the enzyme was measured by measuring ornithine and using the standard curve for different concentrations of ornithine, which ranged from 0.1-0.1M). The effectiveness of arginine was expressed in mmol of ornithine removed from arginine per millilitre (ornithine/min/ml). The specific activity of the enzyme. It is the amount of ornithine removed from arginine in mmol/min/mg protein. [19].

2.3.2 Determination of Protein Concentration

According to the Lowry method (1973), protein concentration was determined using a follen regent. The standard curve of bovine serum albumin (BSA) was used at different concentrations to find the attention of the unknown protein [20].

2.3 Purification of Arginase

Three steps were used to isolate and purify arginase produced from serum diabetes mellitus type two. The protein was precipitated, through a gradual addition of ammonium sulfate, at a saturation rate of 65% to 45 ml of serum for patients with diabetes mellitus type-2. At a temperature of 4 ° C, the addition was slow, using slow stirring and a magnetic stirrer. Leave the precipitate for 24 hours at a temperature of 4 °C. Then the precipitate was separated from the filtrate using a refrigerated centrifuge at 300xg for 20 minutes [21]. Arginase activity and protein concentration were measured in this step.

2.3.1 Dialysis

Membrane sorting is the process of separating small particles from large particles. The membrane sorting process is carried out at a temperature of 4 °C [22]. Arginase activity and protein concentration were measured in this step.

2.3.2 Ion exchange chromatography DEAE-cellulose

DEAE-Cellulose column was used to pass the enzyme through (2.5 cmx35cm). Sodium chloride (NaCl) with a gradual concentration (0.1-1 M) was used for sequential filtration. Each fraction had a flow rate of 1 ml/ minute. Using a U.V. Spectrophotometer, the absorbance of each fraction was estimated at 280 nm. The activity of arginase was measured in each fraction [23]. Arginase activity was measured in this step, and the arginase-active fractions were pooled and held for further steps.

2.3.3 Gel filtration Chromatography

The partially purified arginase produced from the ion exchange technique was added to the column (2.5cm x 50cm) containing Sephadex G-100 at the height of 45 cm. The elution rate was 2 ml/fraction. The optical density of each fraction was measured at 280 nm [24]. Arginase activity in the protein peaks was measured, and the active fractions were collected to further steps.

2.3.4 Determination of the molecular weight of arginase

The molecular weight of arginase was determined by the gel filtration chromatography technique. Sephadex G-100 column (2.5cm x 50cm) was used. many molecular weight markers (204-2000000) Daltons were passed through this column with the arginase. At the same rate, flow 2ml/ fraction. The absorbance was measured at 280 nm [24].

2.4 Study of the optimum conditions for the activity of purified arginase.

Optimum conditions to measure the activity of purified arginase from the serum of patients with type II diabetes were estimated. Several successive experiments were conducted to determine these conditions. The maximum arginase activity was measured using different amounts of serum ranging from 50-to 300 µL. Buffer solutions are characterized by their resistance to any change in the concentration of hydrogen ions when adding large quantities of A small amount of acid or base is added to the solution. The best value pH for the buffer solution and different pH values were estimated from 8-to 11 to determine the optimum pH value at the enzyme showed maximum activity. Also, study the effect of the buffer solution's concentration on the enzyme activity; the buffer solution was used sodium barbitone, with concentrations ranging (from 25-to 150) mM. Furthermore, the optimum temperature of the reaction was also determined by using various temperatures ranging from 25-to 50 oC. The relationship between the incubation period (reaction time) and maximum enzyme activity was studied using a periodic test. Incubation starts from (15-75) minutes to determine the optimal period to complete the enzymatic reaction between a substance (arginine) and arginase. In addition, different concentrations of the substrate (50-300) mM were used to estimate the optimum substrate concentration for the maximum activity of arginase.

2.5 Statistical Analysis

The results in this study were expressed as mean ± standard deviation (S.D.). The results were analyzed using a statistical program (SPSS, version 25). Mean the test was used for calculating the mean ± standard deviation (S.D.) of arginase molecular weight.

3. Results and Discussion

The results in Table 1 show the summary purification of arginase isolated from the serum of patients with type 2 diabetes. The specific activity of arginase in ion exchange was 4.6 (U/mg) with the number of purification 7.8 folds and a recovery of 52.2% (Fig. 1). and increased more in the gel filtration technique, where the specific activity of arginase was 11.4 (U/mg) and several fractions to 19 folds with a recovery of 59.2% (Fig. 2)

Table 1: Purification steps of arginase produced from serum patients with diabetes mellitus type 2(DMT2)

Purification steps	Volume (ml)	Protein concentration (mg/ml)	Activity (U/ml)	Total activity (U/ml)	Specific activity (U/mg)	Purification folds	Recovery %
The crude enzyme (Serum)	45	61	36	1620	0.6	1	100
After Ammonium sulfate precipitation 65%	25	43	48	1200	1.1	1.8	74
After Dialysis	28	23	44	1232	1.9	3.2	76
Ion exchange chromatography DEAE-cellulose/Elution/ fraction no, (65-78)	13	14.2	65	845	4.6	7.8	52.2
Sephadex G-100 (Gel filtration) Elution volume (20-30) ml	12	7.0	80	960	11.4	19.0	59.2

The approximate molecular weight of arginase was estimated using the gel filtration technique, where it appeared, as shown in Figure 2, in two peaks, the first peak contains the high activity of arginase, and it was collected and later used in subsequent experiments to study the optimal conditions for the work of purified arginase. In contrast, the second peak did not show any activity of arginase, so it has been neglected. The results shown in Table 2 and Figure 3 show

the approximate molecular weight of arginase, where it was found that the approximate molecular weight of arginase equals 96050.67 ± 1414.2 Ddaltons compared to the standard marks (Fig. 2ure 2). The result was similar to other studies. It was found that the molecular weight of arginase isolated from patients with type 2 diabetes is equal to 94,000 [14]. As well as, the molecular weight of the result arginase isolated from human blood and liver cells is similar (107000) Dalton [15]. And the molecular weight of arginine purified from human red blood cells is (105000) daltons [16]. The molecular weight of purified arginase was different from other studies and from various sources. The molecular weight of arginase isolated from bacteria was (191,000) Daltons [17], and the molecular weight of arginase isolated from kidney patients was equal to (245,000) Daltons [5]. As well as the molecular weight of arginase isolated from soybean (240000) Daltons [25] and therefore the reason for this difference may be the methods used in the separation and the source of the enzyme

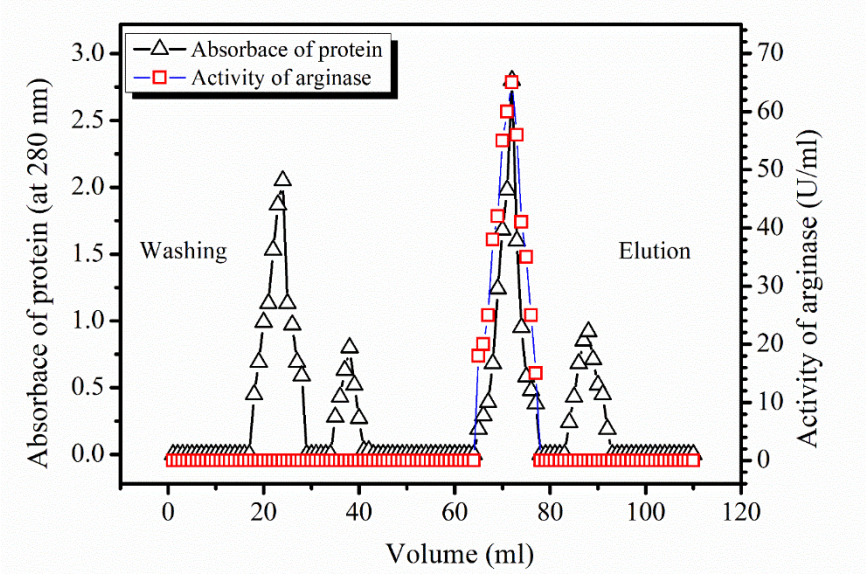

Figure 1: Ion exchange chromatography DEAD-Cellulose column (2.5cm x35cm) for arginase produce from serum diabetes mellitus type two (DMT2) with a fraction at a 1 ml/minute flow rate.

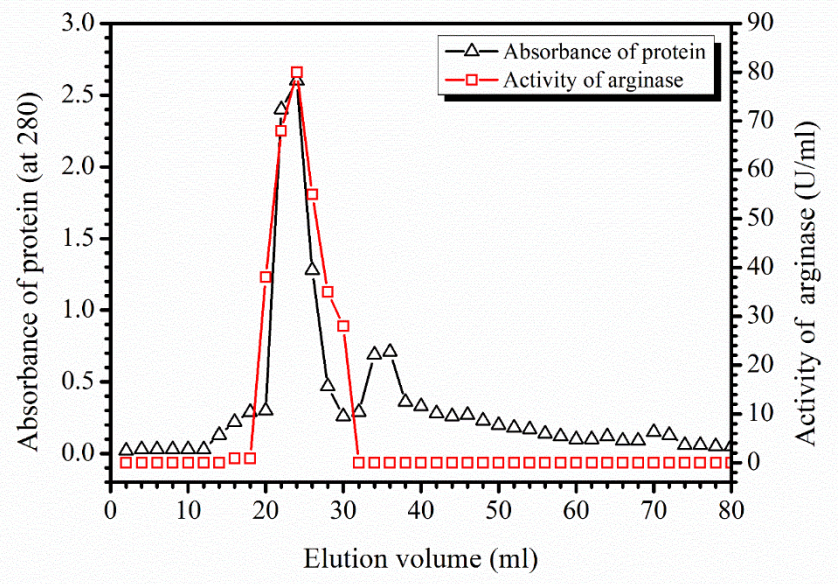

Figure 2: Gel filtration chromatography for arginase from serum diabetes mellitus type 2 (DMT2) using Sephadex G-100 column (2.5cmx50 cm).

Table 2: Log molecular weight of the purified rginase from serum diabetes mellitus type 2 (DMT2) using Sephadex G-100 column (2.5cm x50 cm).

The molecular weight of markers and arginase (Dalton)	Log of M.Wt	Elution volume (ml)
Blue dextran/ 2000000	6.3	14
Hexo kinase/ 100000	5	20
Unknown protein (arginase (mean±SD)/ *96050.67 ± 1414.2	*4.9825	24
Bovine serum albumin (BSA)/ 67000	4.82	66
α –amylase/ 58000	4.76	74
Egg albumin/ 45000	4.65	78
Pepsin/ 36000	4.55	90
Insulin/ 5750	3.75	190
Tryptophan/ 204	2.3	220

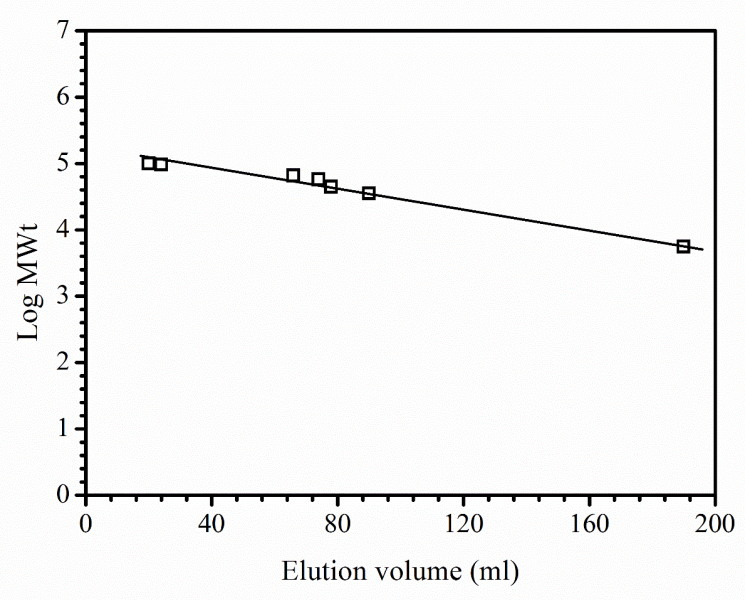

Figure 3: Log molecular weight of the purified Arginase produced from serum diabetes mellitus type 2 (DMT2) using Sephadex G-100 column (2.5cm x50 cm), with a 2 ml/fraction flow rate.1: Hexokinase 2: arginase 3: Bovine serum albumin,4: α –amylase,5: Egg albumin,6: Pepsin,7: Insulin.

Optimal conditions for partially purified arginase were studied in type 2 diabetic patients, As shown in Figure (4). The highest arginase activity was obtained with a volume of 250 µl of the enzyme, which is in agreement with Z. Porembska and M. Kedra [19]. As shown in Figure 5, the highest activity of arginine was at pH 9.5, which is in agreement with J.H.Kang and Y.D. Cho [25]. Figure (6) showed that the highest arginase activity was at a concentration of 100 mM. Figure 7 shows the highest activity of arginine at a temperature of 37 °C. It was found that the optimum temperature is 40°C [26]. Figure (8) also showed that the highest activity of arginase was in the incubation time of 45 minutes. Figure (9) shows that the highest arginase activity was at a concentration of 200 mM of arginine, which acts as a base material, which agrees with [19].

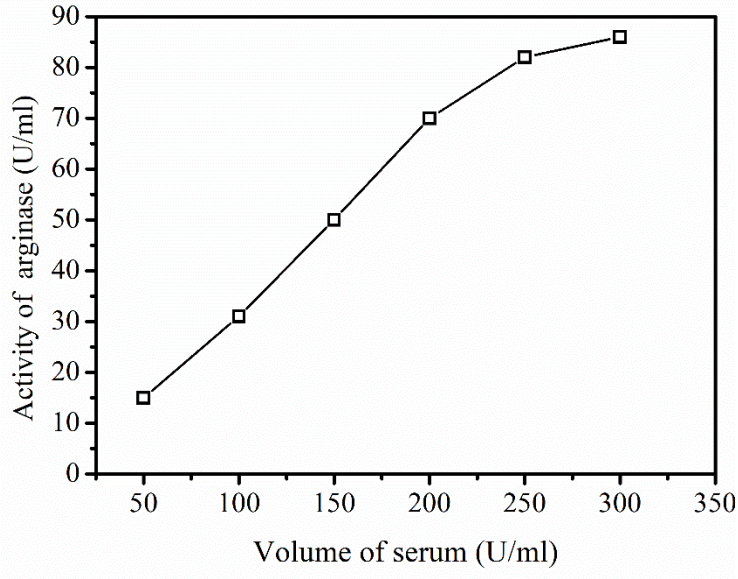

Figure 4: The effect of different volumes of purified arginine (protein) on arginase activity purified from patients with diabetes mellitus type 2.

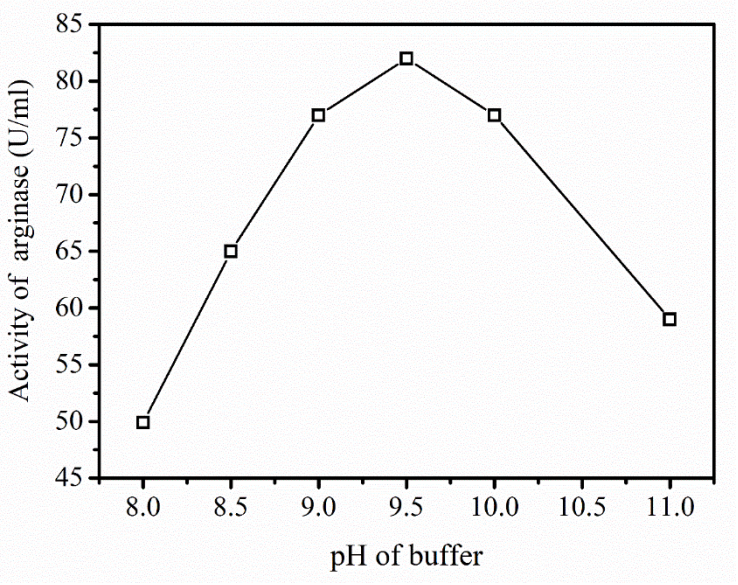

Figure 5: The effect of different pH values for the buffer on the activity of arginase purified from patients with diabetes mellitus type 2.

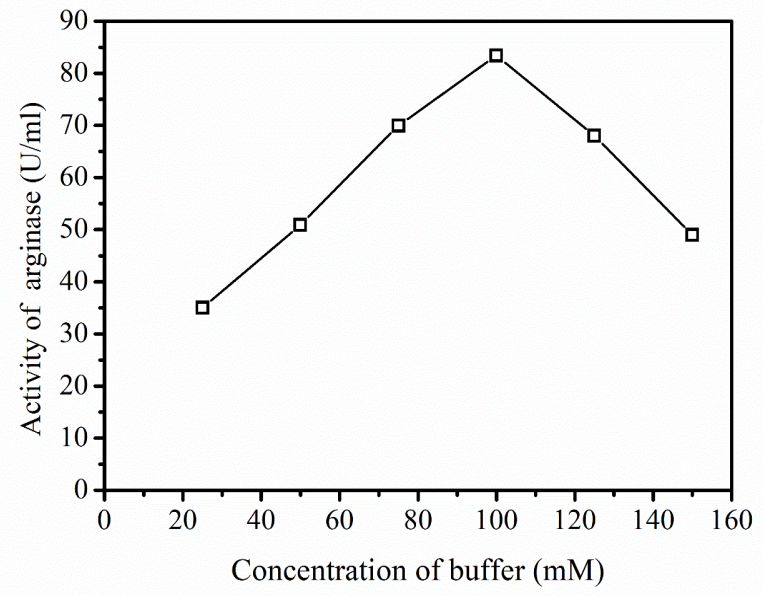

Figure6: The effect of different concentrations of buffer solution on the activity of arginase purified from patients with diabetes mellitus type 2.

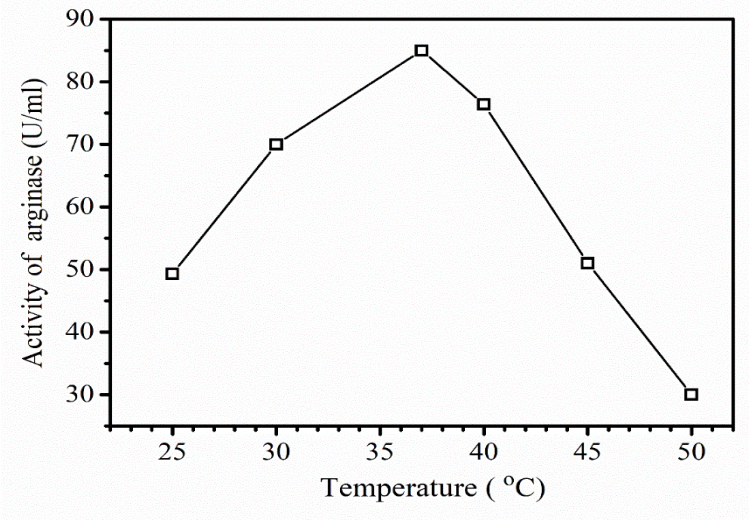

Figure7: The effect of different temperatures on the activity of arginase purified from patients with diabetes mellitus type 2.

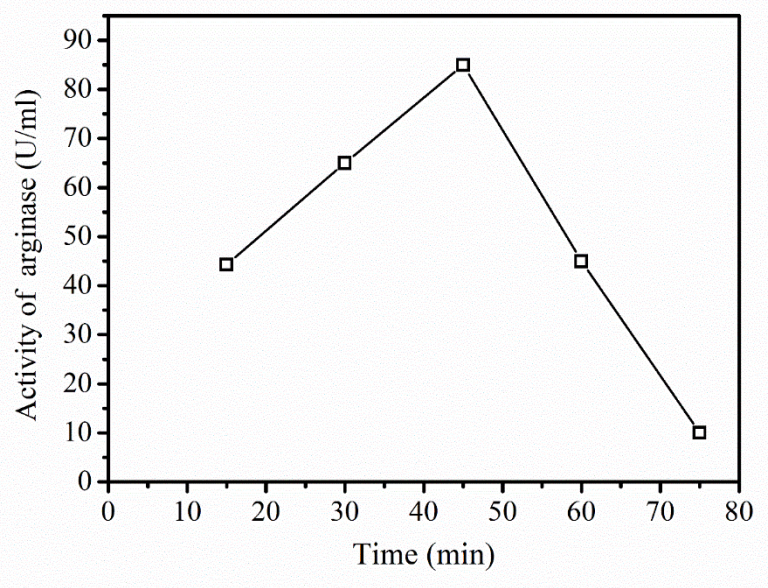

Figure 8: The effect of the different incubation periods (reaction time) arginase the activase purified from patients with diabetes mellitus type 2.

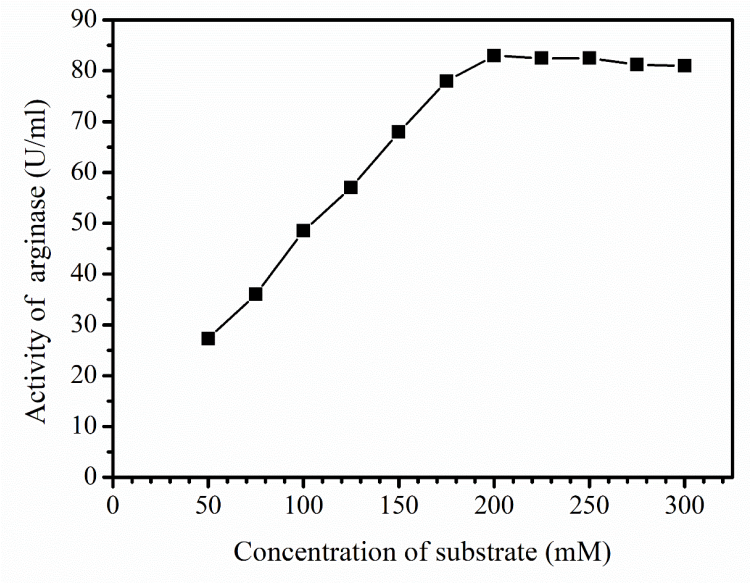

Figure 9: The effect of different arginine concentrations (substrate) on arginase activity purified from patients with diabetes mellitus type 2.

Table 3 shows the optimal conditions reached during this study to measure the activity of arginine purified from serum patients diabetic type 2.

Table 3: The components of an ideal reaction mixture for measuring the activity of arginine purified from serum patients with diabetic type 2.

Source of enzyme	The volume of an arginase (enzyme) (µL)	values of pH	The concentration of buffer solution (Mm)	Temperature (^{o}C)	Reaction time (min.)	concentrations of arginine (substrate)(Mm)
Serum	250	9.5	100	37	45	200

4. Conclusions

The activity and properties of arginine are differ according to the source of the enzyme that purified from it. The specific activity of unpurified arginine equals to 0.6 and found that the specific activity was increased by 19 times when purified, where the specific activity was equal to 11.4 (U/mg). The period of purifications was 19 times and the recovery was 59.2%. The molecular weight of purified arginase is 96050 ± 1414.2 daltons. Furthermore, the purified arginase showed optimal conditions for the enzyme to work. The highest activity of purified arginase was 250 µl of serum as the source of the enzyme, 100 mM of sodium barbitone solution, pH = 9.5, incubation time of 45 min at 37 °C, and 200 mM of arginine as a substrate for arginase.

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دراسة خصائص الأرجينيز المنقى جزئياً من مصل مرضى السكري من النوع الثاني (DMT2) في محافظة نينوى

رؤا وليد الرمضاني، زهراء محمد علي حمودات

قسم الكيمياء، كلية العلوم، جامعة الموصل، العراق

المستخلص

يلعب Arginase دورًا أساسيًا في داء السكري من النوع 2 (DMT2). هدفت الدراسة إلى عزل الأرجينز المصل وتنقيته جزئياً من مصل مرضى السكري من النوع الثاني ، وتقدير وزنه الجزيئي ، وتحديد الظروف المثلى لعمل الإنزيم شملت الدراسة 35 مريضاً من كلا الجنسين (16 ذكور و 19 إناث). تم استخدام ثلاث خطوات تنقية لتنقية الأرجينز: ترسيب البروتين بكبريتات الأمونيوم عند تشبع 65٪ ، كروماتوغرافيا التبادل الأيوني DEAE- السليلوز ، وتقنية نثر الهلام Sephadex G-100. أظهرت نتائج الدراسة أن النشاط النوعي للأرجينين غير المنقى يساوي 0.6 ، وأن النشاط النوعي زاد 19 مرة عند تنقيته ، حيث كان النشاط النوعي يساوي 11.4 (وحدة / ملغم). كان عدد مرات التنقية 19 ضعفًا واسترداد 59.2 ٪. ويساوي الوزن الجزيئي للأرجيناز المنقى 96050 ± 1414.2 دالتون. كان أعلى نشاط للأرجينز المنقى في 250 ميكرولتر من المصل كمصدر للإنزيم ، و 100 ملي مولار من المحلول المنظم صوديوم باربيتون و رقم الهيدروجيني = 9.5 ، وزمن التحضين لمدة 45 دقيقة عند 37 درجة مئوية ، و 200 ملي مولار من الأرجينين كمادة أساس للأنزيم الارجينز. واستنتجت الدراسة إلى أن الفعاليته النوعية للارجينز المنقى من مصل مرضى السكري قد زادت 19 مرة قبل تنقيته. وان خصائص الأرجينز والظروف المثلى لعمله تختلف حسب مصدر الإنزيم المنقى منه.