

Isolation and Partial Purification of Arginase from Sera of Women with Uterine Fibroids

Taghreed U. Mohammd

Eiman A. Abass

Manar A.Salman

Dept. of Chemistry/ College of Education for Pure Sciences (Ibn-AL-Haitham)/
University of Baghdad.

Received in: 12 January 2015, Accepted in: 25 March 2015

Abstract

The first aim of the present study was performed to assay the activity of arginase in sera of women with uterine fibroid.. This study consisted of(50) women with uterine fibroid as patient's group and (30) healthy women as control group. The age ranged between (30-55) years for the two groups. The results showed that highly significant increas ($P < 0.0001$) in the arginase activity in sera of women with uterine fibroid (7.99 ± 0.23) I.U/L is found when compared with healthy group (0.52 ± 0.02) I.U/L.

The second aim was performed to isolate arginase from sera of women with uterine fibroids. The purification is done by addition of ammonium sulfate, dialysis, gel filtration chromatography by using sephadex G-50 and ion exchange chromatography by using DEAE cellulose A-50. The results showed a single band for isoenzymes following steps by using sodium dodecyl sulfate- polyacryl amide gel electrophoresis (SDS-PAGE) .

Key words: Arginase, Uterine fibroid.

Introduction

Uterine fibroids are benign tumors[1] that develop from the muscle tissue of the uterus[2]. They also are called leiomyomas, myomas, fibromyomas, leiomyofibromas. Because the tumor consists of uterine smooth tissue as well as fibroids tissue, the term fibroid does not adequately capture the name of the lesion[3]. These tumors have prevalence ranging from 20% to 40% of the women depending on the age[4].

Studies using both ultra sound and pathologic examination of surgical uterine specimens suggest an overall prevalence of uterine fibroids of over 70%. Uterine fibroids can cause severe problems for women and most of the uterine fibroids do not cause symptoms[5]. The size, shape and location of fibroid can vary greatly[6]. They may be present inside the uterus, on its outer surface or within its wall, or attached to it by a stem[7].

A woman may have only one fibroid or many of varying sizes. Some are no bigger than a pea, while others can grow to the size of a melon or larger, when fibroids are diagnosed. The extent of the disease is determined by comparing the size of the uterus to typical size during pregnancy, for example a large fibroid or multiple fibroids may enlarge the uterus to the same as a six or seven month pregnancy[8].

Arginase (EC.3. 5. 3.1) belongs to the urea hydrolase family which catalyzed the fifth and final step in the urea cycle, converting L-arginine into L-ornithine and the urea[9]. In most mammals, two isozymes of this enzyme exist; the first, Arginase I. functions in the urea cycle, and is located primarily in the cytoplasm of the liver. The second isozyme, Arginase II, has been implicated in the regulation of the arginine/ornithine concentrations in the cell. It is located in mitochondria of several tissues in the body, with most abundance in the kidney[10], prostate, testis, epididymis, seminal plasma vesicles, prostate and human sperm cells. It may be found at lower levels in macrophages, lactating mammary glands and brain[11]. The second isozyme may be found in the absence of other urea cycle enzyme[12].

The first aim of this study was performed to measure arginase activity in sera of women with uterine fibroids comparing to healthy women and the second aim was performed for partial purification and isolation of arginase isoforms from the serum of women with uterine fibroids.

Material and Methods

Subjects

The study is conducted during the period from 1st Dec. 2013 to the end of May 2014 at obstetrics and gynecology department in Baghdad city (Baghdad Teaching Hospital and Al-Yarmuk Teaching Hospital). The study included (50) women with uterine fibroids which were diagnosed by ultrasound and healthy group consisted of (30) women as control group. The age of the two groups ranged (30-55) years.

Specimens Collection and Analysis

Venous blood samples were drawn from each patient then transferred immediately to a clean dry plain tube. After removing the needle, the blood was allowed to clot for at least (10-15) min, at room temperature and then centrifuged for (15) min, at (3500 rpm). Serum is removed and assayed immediately and the samples were stored at 4°C for the purpose of conducting the required measurements.

Methods

1-Assay of Arginase Activity in Serum

Arginase activity was measured in serum according to (Coulombe., 1963)method [13].

Enzyme unit: is the amount of enzyme that catalyses the reaction of 1 μ mol of substrate per minute.

2- Estimation of Protein Concentration

The total protein concentration was estimated in blood serum using a kit provided from a company (SPINREACT) depending Biuret method. (Tiets,N. W. *et al.*, 1995) [14].

3- Purification of Arginase from Serum of Women with Uterine Fibroid

Enzyme purification process had been done using the following steps:

Precipitation by Using Ammonium Sulfate of Concentration 35%

The most frequent used method of protein precipitation is adding of non organic salts like ammonium sulfate, or potassium phosphate. Large amount of water molecules will be bonded to ammonium sulfate when the protein is dissolved in water leading to decrease the amount of water molecules which are interferes with protein, therefore, protein molecules will be concentrated with each other and precipitate [15].

A solid state ammonium sulfate of (0.7gm) was added gradually to a (4mL) serum in a beaker with constant stirring at about (4°C) for one hour till the solution became turbid. Then it was put in a centrifuge at speed (4000 rpm) for (20 min) to split the precipitate from the leach. Finally the precipitate was dissolved in a less amount of buffer (MnCl₂, Tris- HCl pH 8.4).Activity and protein concentration were measured.

Dialysis

Dialysis process had been done by putting the protein solution prepared in the previous step into a tightly wrapped cellophane bag from bottom and then it was wrapped tightly also from its top then the pipe was left in to a container which contains the buffer solution (Na₂CO₃ (0.1M), MnCl₂ (0.005M), Tris-HCl (0.005M)), this process was done at (4°C) with constant stirring using magnetic stirrer. After finishing the dialysis process the final volume of the resultant solution was calculated . The amount of protein and enzyme activity were estimated.

Gel Filtration

Four mL serum taken from dialysis bag was added slowly on the surface of the sephadex G-50 column (20x1.5 cm) and left for five minutes to be absorbed. Ten fractions were collected by bassing buffer solution (Na₂CO₃ (0.1M), MnCl₂ (0.005M), Tris-HCl (0.005M) pH 8.3) through the column .Entire operation was carried out inside a refrigerator. Flow rate (2 mL/min).

Ion Exchange Chromatography

Two mLs of the fresh filtered serum sample were passed through a column of sephadex ion exchange chromatography DEAE-Cellelose A 50 pre-equilibrated (Na₂CO₃ (0.1M), MnCl₂ (0.005M), Tris-HCl (0.005M) pH 8.3) . The sephadex ion exchange column of the size of (20x1.5 cm) containing gradient concentration of potassium chloride (0.1-0.3M/L). Entire operation was carried out inside a refrigerator. Flow rate (2mL/min).

SDS- PAGE Electrophoresis

SDS- polyacrylamide gel electrophoresis (10%) was carried out according to Laemmli *et al* [16]. The purified enzyme was treated with 1% SDS and β -mercaptoethanol for 10 minutes at 100° C and loaded in wells. Samples stacking was done at 10 mM and resolution was carried out at 15 mA constant current. Molecular weight standards were bovine serum albumin (66,000), ovalbumin (45,000), chymotrypsinogen (25,000) (Sigma). After electrophoresis gel was stained in 0.25% w/v Coomassie brilliant blue (R-250) prepared in 50% v/v methanol and 10% v/v acetic acid. The gels were destained by passive diffusion of dye in 50% v/v methanol and 1% acetic acid after changing the destaining solution for 2-3 times.

Statistical Methods

Statistical analysis was used to show the mean and standard error deviation of variables. The significance of difference between mean values was estimated by student T-Test . The probability $p < 0.05$ = significant . $p > 0.05$ = non- significant. ANOVA test was used to show the differences between variables of differentiated groups. The data were processed with the software package SPSS (statistical package for social sciences) Ver.17 and Microsoft Excel version 2007 .

Results and Discussion

Table (1) shows the mean \pm SE and p- value of the arginase activity in sera of women with uterine fibroid and healthy women.

The result in table (1) shows a highly significant increase in the arginase activity in the women with uterine fibroid (7.99 ± 0.23 IU/L) when compared to healthy women (0.52 ± 0.02 IU/L).

Arginase can redirect the metabolism of L-arginine in smooth muscle cells from nitric oxide to L-ornithine and the formation of polyamines and L-proline, which can induce vascular lesion formation by stimulating smooth muscle cell proliferation and collagen deposition. These actions of arginase are further magnified by the suppression of NO release, which serves as a well- recognized inhibitor of smooth muscle cell growth and collagen synthesis[17]. While (Kaplen *et al.*,2012)[18], revealed the increase in arginase activity may limit nitric oxide synthase (NOS) activity and lead to decrease of the inhibitor effect on xanthine oxidase activity. In this case, it results in more superoxide radical production and tissues damage. As evidenced by the up regulation of arginase in specific disease states, its distribution in vagina, and its modulation by sex steroid hormones, this enzyme may also tissue growth fibrosis, and immune function[19]. The recent study demonstrated that arginase activity may be attributed to change in permeability of fibroid cell as a result of many biochemical changes in cell surface, that included changes in the appearance of cell surface such as variation in glycolipid and mucin[20].

Partial Purification of Arginase from Sera of Women with Uterine Fibroids

Table(2) summarises the result of isolation and partial purification of arginase and its isoenzymes from women sera with uterine fibroids which carried out through steps. In the first step, the proteins precipitate by using ammonium sulfate salt in concentration of (35%) for the concentration of enzyme and purified, degree of purity was obtained (9.9) fold and excess salt was eliminated during dialysis process by Na_2CO_3 (0.1 M), MnCl_2 (0.005 M), Tris.HCl (0.005 M) with pH= 8.3. The degree of arginase purity reached to(3.337) fold in this stage with enzyme yield (92.46%) . The third step included purification of enzyme by gel

filtration using (Sephadex G-50) which the degree of arginase purity reaches to (5.822) fold which enzyme yields (47.60%) in this stage ,as shown in figure (1).

The purification by ion exchange chromatography is among the significant approaches to isolate and purify the enzyme that is based on the charge difference so the enzyme was purified by using DEAE-cellulose A50. Four isoenzymes were obtained as illustrated in figure (2) and variant purity degree as the degree of purification of I (4.407) fold and II (4.867) fold for the isoenzymes III, IV (5.834), (5.696) folds respectively, as illustrated in table (2).

The literatures indicate that two isoenzymes were separated from adult human's liver by using CM-cellulose chromatography [21] and the two isoenzymes of the arginase from rat's liver [22] by using DEAE-cellulose. Tarrab *et al.*, 1974) isolated three isoenzymes from the rat's liver using CM-cellulose chromatography[23].

Gel electrophoresis was used to confirm the purity of the isolated isoenzymes by using sodium dodecyl sulfate- polyacryl amide gel electrophoresis (SDS-PAGA) which appeared as single band.

Conclusion

These data suggest that an isolation and partial purification of isoenzymes of arginase from sera of women with uterine fibroids by gel filtration and ion exchange chromatography four isoenzymes were obtained (I,II,III,IV).

References

1. Leppert, P.; Fouany, M.; and Segars, J. H. (2013). "Fibroids". 1stEd. John Wiley & Sons, Ltd.pp 1-11.
2. Nivethithai, P.; Nikhat, S. R.; and Rajesh, B. V.(2010)." Uterine fibroids".A review. Indian J. Pharmact., 3(1): 6-11.
3. Levy, B.; Mukherjee, T.;and Hirschornk. (2000). Molecular cytogenetic analysis of uterine leiomyoma and leiomyosarcoma by comparative genomic hybridization. Cancer Genetcytogenet., 121: 1-8.
4. Duhan, N.;and Sirhiwal, D. (2010). Uterine myomas revisited. Eur. J. Obstet Gynecol. Repro. Biol., 152: 119-25.
5. Lgnacio; E. A.; and Venbrux, A. C. (2012). Women's health in interventional radiology, 5th Ed. Springer., 4-6.
6. Acton, Q. A.(2012). Leiomyomas: New insights for the healthcare professional, 2012th Ed. Scholarly, 2.
7. Goodwin, T. M.; Montaro, M. N.; Muderspash, L.; Paulson, R.;and Roy, S. (2010) . "Management of common problems in obstetrics and gynecology", 5th Ed. Wiley& Sons.291-292.
8. Ficalora, R. D. (2013) . "Myoclinic internal medicine board review", 10th Ed. Oxford University Press.712-714.
9. Nelson, D. L.;and Cox, M. M. (2013) . "Lehningerprinciples of biochemistry" , 6th Ed. W. H. freeman & Company, 704-706.
10. Stephen, D. C.; Hong, Y.; Wayne, W.G.; Rita, M. K.; Paul, Y.;and Ramaswamy, K. I. (2004) . Arginase I and II: do their functions overlap?. Mol. Gen. Metab., 81: 38-44.
11. Elgün, S.; Kacmaz, M.; Sen, I.and Durak, I. (2000) . Seminal arginase activity in infertility. Springer, 28: 20-23.

12. Di Costanzo, L.; Moulin, M.; Haertlein, M.; Meilleur, F. and Christianson, D. W. (2007) . Expression, purification, assay, and crystal structure of perpetuated human arginase I archives of biochemistry and biophysics, 465(1): 82-9.
13. Coulombe, J. J.; and Favreau, L. (1963). A new simple semimicro method for colorimetric determination of urea. Clin. Chem., 9-102.
14. Tiets, N. W. (1995) . "Clinical guide to laboratory". Tests, 3th Ed. AACC.
15. Robyt, J.F.; and White, B.J. (1987) . Biochemical Techniques: Theory and practice, Books/cole publishing company, Belmont, CA.
16. Laemmli, U. K. (1970) Cleavage of structural proteins during the assemble of the head of the bacteriophage T₄. Nature., 227:680-685.
17. Durante, W.; Johnson, F. K. and Johnson, R. (2007). Arginase: A critical regulator of nitric oxide synthesis and vascular function. Clin. Exp. Pharmacol. Physiol., 34(9): 906-911.
18. Kaplan, I.; Aydin, Y.; Bilen, Y.; Gen, C. F.; Keles, M. S.; and Eroglu, A. (2012) . The evaluation of plasma arginine, arginase, and nitric oxide levels in patients with esophageal cancer, 42(3): 403-409.
19. Kim, N. N.; Christianson, D. W.; and Traish, A. M. (2004). Role of arginase in the male and female sexual Aromsal. Response. J. Nutr., 134(10): 2873-2879.
20. العباجي، صبا زكي؛ عمر، دعاء حسين. 2013. دراسة كيميائية حيوية لأنزيم الرايبونوكليز RNase . في مصل دم وأنسجة المصابات بأورام الرحم. مجلة علوم الراقدين، 24(5): 64-80.
21. Bascur, L.; Cabello, J.; Veliz, M.; and Gonzales, A. (1970). Biochim. Biophys. Acta., 128: 149-154.
22. Gasiorowska, I.; Poremska, Z.; Jachimowic, Z. J.; and Machnacka, I. (1970). Acta. Biochim. Pol., 17: 19-30.
23. Tarrab, R.; Rodriguez, J.; Huitron, C.; Palaciros, R.; and Soberon, G. (1974) . Eur. J. Biochem., 49: 457-468.

Table No. (1): mean±SE of arginase activity in the sera of women with uterine fibroid and healthy women.

Arginase(I.U/L)	N	mean± SE	p-value
Uterine fibroid women	50	7.99 ± 0.23	<0.0001
Healthy women	30	0.52 ± 0.02	

Table No. (2): Isolation and purification of arginase isoenzymes from uterine fibroids women.

Step	Elute (mL)	Activity I.U/L	Total activity (IU)	Prot Conc. mg/L	Total port (mg)	Specific activity IU/mg	Purification Fold	Yield %
Crude serum	4	7.30	0.0292	840	3.360	0.00869	1	100
Ammonium sulphate	2	9.9	0.0198	529	1.587	0.0187	2.151	67.80
Dialysis	2	13.94	0.027	480	1.440	0.0290	3.337	92.46
Sephadex G-50	2	6.99	0.0139	138	276	0.0506	5.822	47.60
Ion-exchange								
Iso.I	2	4.6	0.00920	120	240	0.0383	4.407	31.50
Iso.II	2	5.21	0.0104	123	246	0.0423	4.867	35.63
Iso.III	2	5.99	0.0119	118	236	0.0507	5.834	40.75
Iso.IV	2	5.8	0.0116	117	234	0.0495	5.696	39.72

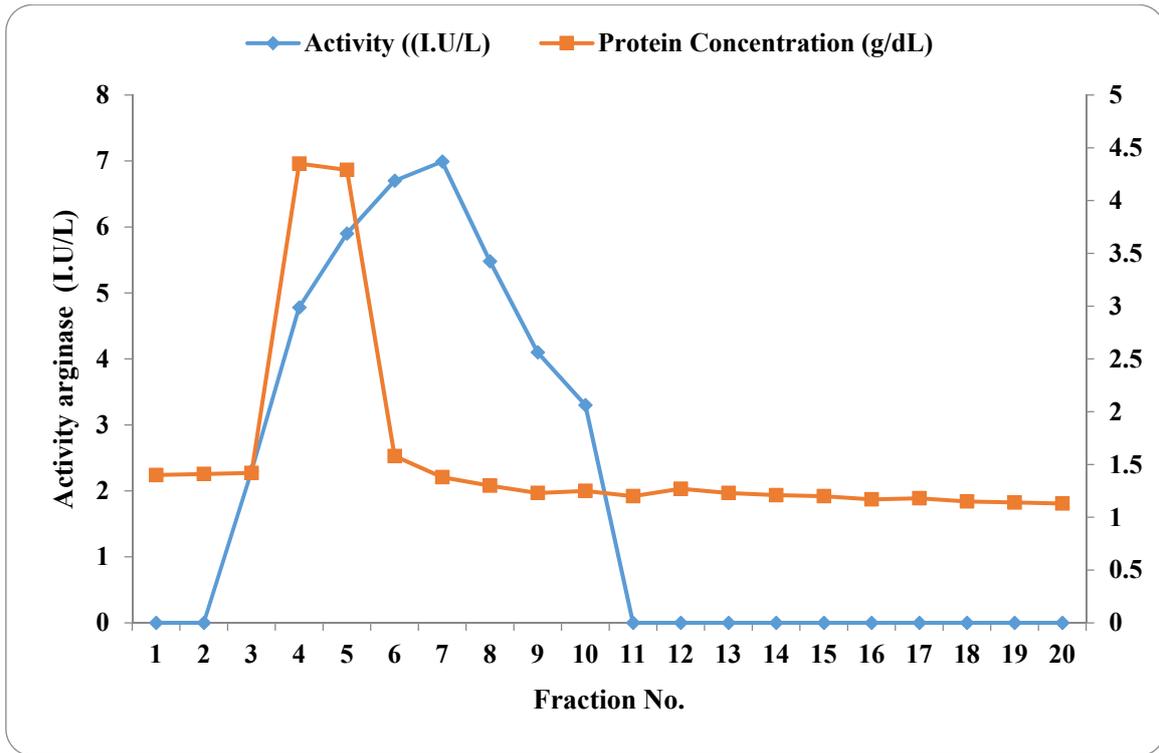


Figure No.(1):Purification of arginase from uterine fibroids women by using chromatography Sephadex G-50.

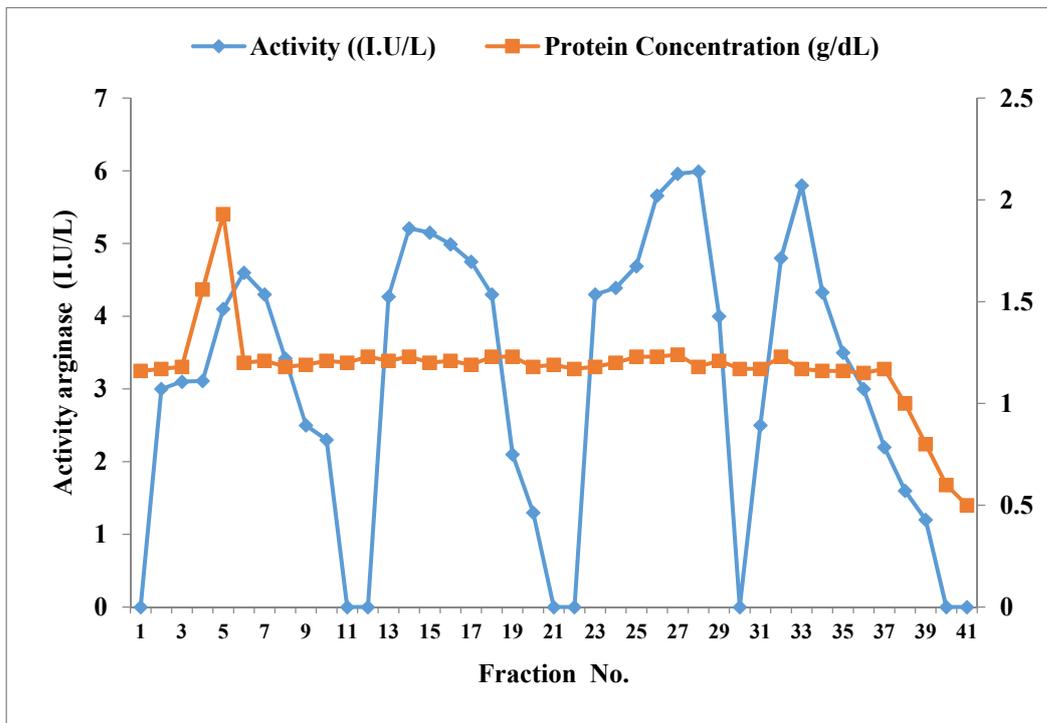


Figure No. (2): Isolation of arginase isoenzymes from srea uterine fibroids women by using chromatography Ion exchange DEAE- cellulose A50.

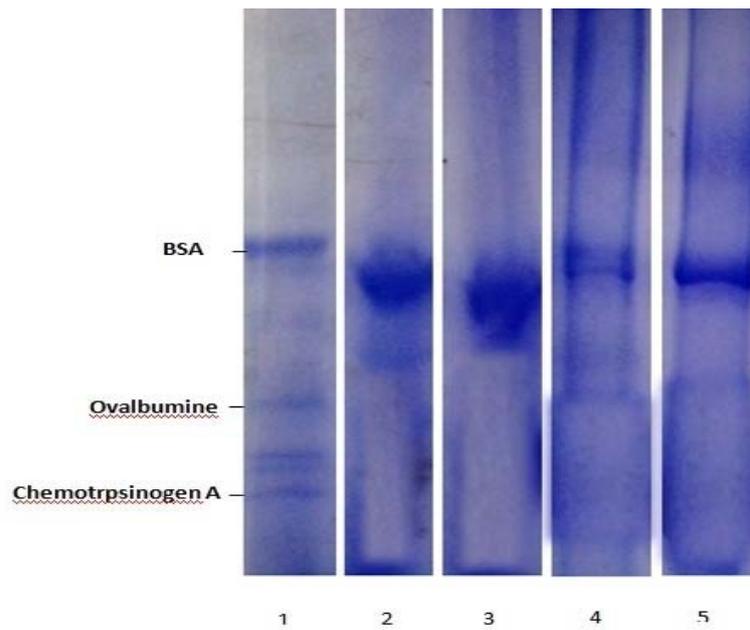


Figure No.(3):Electrophoresis of isolation arginase

فصل وتنقية جزئية لأنزيم الأرجينيز من أمصال النساء المصابات بتليف الرحم

تغريد علوم محمد
أيمان عبد علي عباس
منار عباس سلمان

قسم الكيمياء/ كلية التربية للعلوم الصرفة (أبن الهيثم) – جامعة بغداد

استلم البحث في: 12 كانون الثاني 2015، قبل البحث في 25 آذار 2015

الخلاصة

هدفت الدراسة الحالية أولاً إلى قياس فعالية أنزيم الأرجينيز من أمصال النساء المصابات بتليف الرحم. تضمنت هذه الدراسة (50) امرأة من المصابات بتليف الرحم و (30) امرأة سليمة بوصفهم مجموعة مقارنة، تراوحت أعمارهن بين (30-55) سنة في المجموعتين. بينت النتائج وجود ارتفاع معنوي ($P < 0.0001$) في فعالية أنزيم الأرجينيز في النساء المصابات بتليف الرحم (7.99 ± 0.23) وحدة عالمية/لتر، بينما كانت معدل فعاليته (0.52 ± 0.02) وحدة عالمية/لتر في الأصحاء.

أما الهدف الثاني هو فصل وتنقية متناظرات انزيم الارجينيز من امصال النساء المصابات بتليف الرحم من خلال الترسيب بكبريتات الامونيوم والديلزة وبأستعمال تقنية كروماتوغرافيا الترشيح الهلامي sephadex G-50 وكروماتوغرافيا التبادل الأيوني DEAE cellulose-50 تم فصل أربع متناظرات أنزيمية تختلف في درجة تنقيتها. وعند استعمال طريقة الفصل بالترحيل الكهربائي على هلام SDS-PAGE بتركيز 10% وبأستعمال صبغة Coomassie blue R250 ظهرت حزمة منفردة لكل متناظر.

الكلمات المفتاحية: أنزيم الأرجينيز، تليف الرحم.