



Isolation of Trypsin from Serum of pancreatic Cancer Patients and determination some biochemical parameters

Layla A. Mustafa , Zena A. Mohammad

Department of Chemistry , College of Science , University of Mosul , Mosul , Iraq

ARTICLE INFO.

Article history:

-Received: 2 / 11 / 2017

-Accepted: 23 / 1 / 2018

-Available online: / / 2018

Keywords: Trypsin, Pancreatic Cancer.

Corresponding Author:

Name: Zena A. Mohammad

E-mail:

Zeena.ahmeed98@gmail.com

Tel:

Affiliation:

Abstract

The research included isolation and purification of Trypsin from serum of patient with pancreatic cancer, and estimation its molecular weight by using the two techniques of gel filtration (using sephadex G-50) and electrophoresis (using SDS). Therefore; it was noted that the proteinous peak isolated from ammonium sulfate solution is saturated (65%) of the serum by using the two techniques gel filtration and electrophoresis had apparent molecular weight (27000) Dalton. The study included (40) patients with pancreas cancer their ages ranged between (30-70) years who attended Al-Jumhuri and Atomic Medicine Hospitals in Mosul; the laboratory examination included estimation levels of: Trypsin, Gamma Glutamyl transferase, Amylase, Paraoxonase, Glutathione, Sialic acid and Ceruloplasmin. Results showed that level of Trypsin and Amylase in the serum decreased significantly in patients with pancreatic cancer in comparison with the control group, but levels of Gamma Glutamyl transferase, paraoxonase, Glutathione, Sialic acid and Ceruloplasmin increased significantly in patients with pancreatic cancer in comparison with the control group.

Introduction

Trypsin (EC 3.4.21.4) has a molecular of the range (22-23) KD of high specific in analysis bond of peptide in which carboxyl group belongs to Arginine or lysine [1]. The enzyme is secreted from cells of the pancreas in from of ineffective zymogene, then the enzyme is used in different researching industries and applications such as reaction of preparing the pieces of peptide during analysis of the protein and makes it easy to absorb the large proteins by breaking them and changing into smaller proteins; whereas Trypsin is frustrated inversely by α -1-protinase inhibitor in case of no need because continuation the work of trypsin leads to cause damage to tissues of the cell [2]. Also, Trypsin works at hydrogenic foundation (pH= 8) and it does not work at a certain temperature and certain pH whereas the enzyme is stable for (2-3) years at pH=3 and temperature (-20) distant humidity. Trypsinogen the ineffective from of the enzyme the molecular weight is (24) Dalton changes into the effective form, whereas Trypsin – its molecular is (23.8) Dalton in displacement of the hexadecimal peptide from the amino end through breaking bond of lysine residue at the position (15) by action of Entrokinase which is secreted from mucosa of the

small intestine [3]. Pancreatic Cancer is called the silent cancer, it originated as an irregular work of the genes in tissues of the pancreas which produce abnormal cells caused by environmental or hereditary factors, therefore it is the most truculent dangerous species because it is difficult in diagnosis in its initial stages. Also, Its symptoms are ambiguous un clear in the early stages of the disease, whereas they interfere with symptoms of other diseases such as pancreatitis or inflammation of the stomach. Then, because the pancreas is situated behind the stomach, so diagnosis of the disease is difficult in the initial stages. thus, the tumor grows up and spread before its diagnosis in an early stage in most cases it can discovered but the cancer has advanced for an early stage, in other cases it has spread reaching other members of the body[4,5] because there is no previous study in Iraq about isolation the enzyme from serum of pancreas cancer, so the aim of the researchis isolation trypsin and estimation its molecular weight by using the two techniques gel filtration and electrophoresis, the studying levels of some biological variables that subject to disorder because of the disease casualty.

Materials and Methods**Samples:**

For studying the biochemical parameters (40) blood samples were collected for the patient caused by pancreatic cancer their ages ranged (30-70 years old) who consult Al-Jumhuri Hospital and Atomic Medicine Hospital in Mosul, to be compared with (40) blood sample of healthy persons uncaused by the disease with the same age category, whereas the Information related to the patients were written down according to a questionnaire form specialized for this purpose.

Preparation of blood samples:

Blood samples were collected (Patient and Control) in dry and clean tubes and put in a water bath at (37) centigrade degree for (15) minutes ; them the clotted part was isolated from the pure solution by using the centrifugal device at speed (3000 rpm) for (15) minutes after which the pure part which represents the serum was taken and maintained at (-20) centigrade degree after isolation directly.

Precipitation and Isolation of the enzyme:

The precipitation of Trypsin from serum patients with pancreatic cancer using ammonium sulphate and saturated (65%)[6].

Estimation the Approximate Molecular Weight of the Trypsin:**-By Gel filtration chromatography technique:**

This technique was used for isolation and estimation its approximate molecular weight [7]. the material gel was used sephadex (G-50) in packing the separation column of the dimensions (115x1.8 cm) then the gel was put at height of (90 ml) . then , the protein isolated compound, the unknown molecular weight was dealt with, also the protein contend was followed up through reading intensity of absorption at the wave length (280 nm) , there after the material resulting from gel precipitation technique was dried by using lyophilizer technique to get this protein material in form of dry powder.

-By SDS –PAGE Gel Electrophoresis:

Electrophoresis technique was used by using SDS to isolate the charged particles under electrical field according to the method followed by the researcher [8].

Trypsin activity was measured using method of Erlanger et.al. [9] and Modified by Benjakul et.al. [10] who used BAPNA as a substrate , there after the intensity of absorbs light was read at (410 nm).

Gamma Glutamyl transferease activity was measured using method by Szasz et.al. [11] who used L-gamma – glutamyl –4-nitro anilide as a substrate, the reaction of the substrate and glycyglycine produces p-Nitro aniline and giving high absorption at (450 nm).

Amylase activity was measured by using the Kit method of type from French Biolabo company. whereas principle of the reaction depends on the method E-PNPG7[12]. it is a sensitive and accurate method, as the speed of formation PNP is directly

proportional of the enzymes activity in the sample and is measured at wave length of (405 nm).

Paraoxonase activity was measured by using method of Furlong et.al. [13] . the principle of the method depends on the speed of paraoxone hydrolyses to paranitrophenol and diethyl phosphoric acid and it is depended on estimation of paranitrophenol concentration resulting at (450 nm).

Glutathione concentration was measured by using modified method of Sedlak and Lindsay [14]. Absorption intensity is measured at the wave length of (412 nm), the concentration of the formed product depends on the amount of Glutathione present in the serum

Sialic acid concentration was measured by using the modified method of the Miettinen and Luukkainen [15] , and intensity of absorbability can be red at wave lengt(580nm).

Ceruloplasmin concentration was measured by using modified method of Menden et.al. [16]. The principle of the method depends on the activity of ceruloplasmin in oxidation the paraphenyl diamin colorless to a purple – bluish compound. the absorption intensity is measured at (525 nm).

Statistical Analysis: The results were analyzed statistically by using T-test at a level of probability (P values \leq 0.05) for comparison between the two groups of the patients and control. also biochemical parameters values were described by using each of mean and standard error.

Result and Discussion

Gel filtration chromatography technique was used for isolation and estimation its approximate molecular weight [7]. A Shown in Fig. (1) the elution volume of Trypsin solution, collected from gel filtration separation column is (92.6 ml) and this correspond to a molecular weight of (27000 Dalton) when using the Standard Curve Shown in Fig.(2).

As Shown in Fig. (3), the pure enzyme obtained shows a single band when analyzed by acrylamide electrophoresis in the presence of 0.1% Sodium dodecyl sulphate [8] the relative distance of the Trypsin is (5.7cm) and it is clear that the enzyme has a molecular weight equal to (27000 Dalton) when using the standard curve shown in Fig.(4).

All biochemical parameters were compared between control and patients groups as shown in the Table (1). It was showed from the results a significant decrease in the activity of Trypsin in the serum of the patients comparison with the control and this is consistent with Fedial et.al. [17], the low activity of Trypsin in the serum of patient with pancreatic cancer is unclear that may attributed to a certain harm as a result of an acute or chronic pancreatitis that causes erosion the of pancreas and its damage by action of the digestive enzymes secreted by the gland because of the result of the bile duct obstruction jointly on gall bladder stones coming down causing non with drawl of the digestive enzymes produced by the gland [18].

The results observed to significant increase of gamma glutamyl transferease activity in the patients comparison with the control group, this is consistent with Rutetenberg et.al. [19], The enzyme works glutathione to protect the cell from damages and to take off the toxicity of the treating medicine expelling out of the cell them resist the medicines responsible for oxidation [20]

The results indicated also to significant decrease a decrease in the amylase activity in the patients comparison with the control group, enzyme activity is measured in evaluation on the kidney function and diagnosis of disorders in the pancreas . Decrease in amylase activity is attributed to the harms caused to cells of the pancreas producing amylase in case of pancreas fibrosis especially [21].

It was noted also a significant increase in paraoxonase activity in the patient comparison with the control group, the enzyme works as a protective system against the oxidation stress ,whereas In case of cancer oxidation stress increase and production of free radicals, therefore, the enzyme works as a protection system against the oxidation stress, the enzyme is more activity in removing the free radicals

and toxicity of many molecules, laterit works for stability of the cell [22].

It showed also a significant increase in Glutathione concentration in the patients comparison with the control group, Glutathione concentration increases in pancreatic cancer because Glutathione work as a antioxidant , where it works the capture free radical in case of cancer [23].

Also the results showed a significant increase in Sialic acid concentration in serum patients comparison with the control group, Sialic acid concentration increases in pancreatic diseases [24] Increase of sialic acid level is attributed to increase in oxidation stress level ,Sialic acid works the capture the free radical especially H_2O_2 [25].

Finally the results showed a significant increase of ceruloplasmin in the serum of the patients comparison with the control group, ceruloplasmin has been shown to be associated with the changes occurring in the cancer cells and it plays important role to stimulate the proliferation of tumor cells, also copper contributes to produce the free radical indirectly and disorder and destroying of the cells later [26].

Table (1) The Comparison of serum biochemical parameters between patients and control group ($p \leq 0.05$)

Parameter	Mean± Standard error	
	Control(40)	Patients (40)
Trypsin (U/ml)	0.202±0.063	0.129±0.0083*
Gamma glutamyl transferease (U/L)	0.0328±0.0063	0.1294±0.0138**
Amylase (U/L)	70.318±4.28	52.739±10.344*
Paraoxonase (U/ml)	0.6941±0.0448	1.4818±0.1572**
Glutathione ($\mu\text{mol/L}$)	1.868±0.0779	5.885±0.4087**
Sialic acid ($\mu\text{g/ml}$)	35.297±4.338	59.637±2.085**
Ceruloplasmin ($\mu\text{mol/L}$)	0.9678±0.0491	1.91522±0.0552**

Values are presented as mean \pm SE.

**increased significant ($p \leq 0.05$) compared with control .

*decreased significant ($p \leq 0.05$) compared with control .

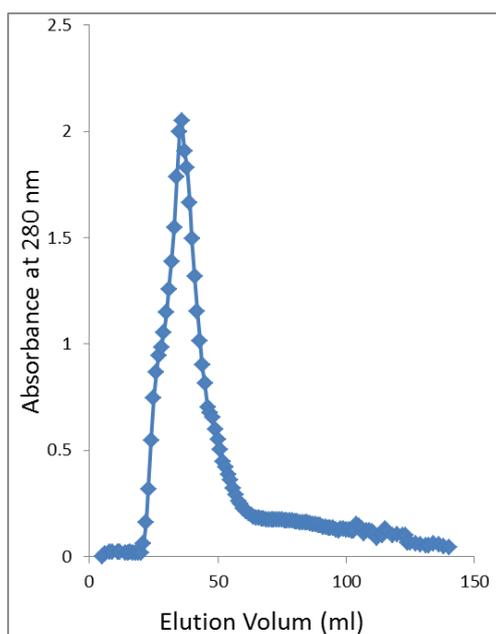


Fig. (1): The peak of Absorbance of Trypsin enzyme Out putting From Gel filtration Column (115 x 1.8)

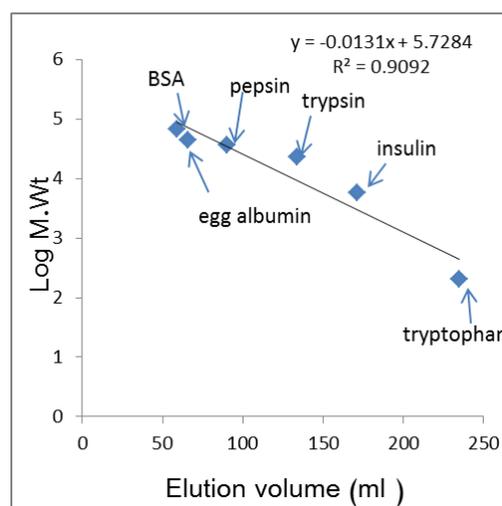


Fig. (2): Calibration Curve used to Determine the Approximate Molecular weight of Trypsin using Gel filtration technique

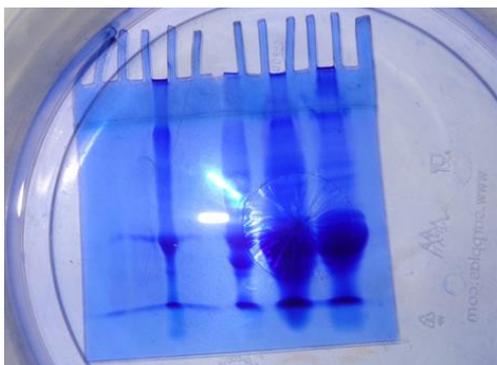


Fig.(3): SDS – PAGE analysis bands of the purified Trypsin and the standard proteins

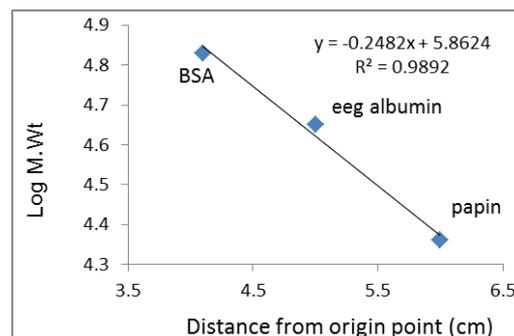


Fig. (4): Calibration Curve used to Determine the Approximate Molecular weight of Trypsin using SDS-PAGE Polyacrylamide Electrophoresis

Reference

- 1- Francisco Javier Castillo, Romon Pacheco, Fernando Luis Garcia, Maria de Los Angeles Navarrete (2005). "Isolation and characterization of Trypsin from pyloric caeca of Monterey Sardine *sardinops sagax caerulea*". comparative biochemistry and physiology, part B 140, 91-98.
- 2- Yasushi Sasai, Hiroshi Kanno, Naoki Doi, Yukinori Yamauchi, Masayuki Kuzuya and Shin-ichi Kondo (2016) "Synthesis and Characterization of Highly Stabilized Polymer – Trypsin conjugates with autolysis resistance " Gifu pharmaceutical university, Japan.
- 3 -Petra Pallagi (2013). "The effects of NA^+/H^+ Exchanger Regulatory factor 1 and Trypsin on Pancreatic Ductal Bicarbonate Secretion".
- 4- Siegel R.L., Miller K.D. and Jemal A.(2015). "cancer statistics". CA Cancer J.Clin.65, 5-29.
- 5-Ali Stunt, (2016), "Hop for the further : Tackling inequalities in Pancreatic cancer care".
- 6- Robyt J.F. and White B.J. (1987). "Biochemical Techniques Theory and practice". Wads Worth Inc. Monterey, California, USA .; 235-236 ,263,269.
- 7- Andrews, P., (1965). The gel filtration behavior of proteins related to their molecular weight over a wide range. J. Biol. Chem. Vol. 96: 595p.
- 8- Laemmli; UK. (1970). "cleavage of structural proteins during the assembly of the head of bacteriophage T4 ".Nature., 227(5259): 680-685.
- 9- Erlanger B.F., Koko wsky N. and Cphen W. (1961). "The Preparation and Properties of trypsin ". Arch. Biochem. Biophys. 95, 271 – 278.
- 10- Benjakul S., Visessanguan W. and Thummarat wasik P. (1999) ."Isolation and characterization of Trypsin inhibitors from some Thai Legume seeds ". J. Food Biochem. 24 ,107 – 12.
- 11- Szasz G., Boone J., Rosalik S.B., Shaw L. M. , Voigtlander K. and Bondar R. J. L.(1976). " Reaction – Rate Method for gamma – glutamyl transferase activity in serum " Clinical Chemistry Vol. 22 No. 12 P. 2051 – 2055.
- 12- Tietz N.W. Text book of clinical chemistry, 3th Ed. C.A. Curtis, E.R. Ashwood W.B. Saunders (1999) P.689 -698 , 1284, 1286.
- 13- Furlong C.E., Richter R.J., Seidel S.L., Costa L.G. and Motulsky A.G. (1989). "Spectrophotometric

assays for the enzymatic hydrolysis of the active metabolic of chlorpyrifos and parathion by plasma paraoxonase / arylesterase" Analytical Biochemistry , Vol. 180 , No.2 , pp. 242 – 247.

14 -Sedlak J. and Lindsay R.H. (1968) ." Analytical biochemistry ". p. 192. Cited by Al- zamyle .(2001).

15 -Miettinen T. and Luukkainen T. (1959). Acta. Chem. Scand. 13(4) : 856-885.

16- Menden E.E., Boiano J.M., Murthy L. and Petering H.G.(1977). "Modification of phenylene diamine oxidase method to permit non-automated ceruloplasmin determination in batches of rat serum or plasma micro samples". Analytical . 10:197 – 204.

17- Fedail S.S., Harvey R.P., Salmon P.R., Brown P. and Read A.E. (1979). "Trypsin and Lactoferrin levels in pure pancreatic juice in patients with pancreatic disease " Gut., 20,983-986.

18 -Tianming Zhang, Feng Gao , Ling Guo , Yan Wang ,Zheng Guo .Chengfei Zhao (2017) . "rs10273639 C/ C Geno type of PRSSI: How can It affect management of pancreatic cancer ? " . Jop. J Pancreas (online) 31; 18 (4): 310 -315.

19- Rutetenberg A.M., Goldbarg J.A., Pineda E.P. (1963). "Serum gamma glutamyl transpeptidase activity in hepatobiliary pancreatic disease". Gastroenterology . 45 : 43 -8.

20- pompella A., Corti A., Paolicchi A., Giommarelli C., Zunino F. (2007)." gamma-glutamyl transferease, redox regulation and cancer drug resistance " Curr. Opin. Pharmacol , 7(4):360-366.

21- Andrea Casadei Gardini, Marita Mariotti, Alessandro Lucchesi, Sara Pini, Martina Valgiusti, Sara Bravaccini, Angelo Del Monte, Marco Angelo Burgio, Giorgia Marisi, Dino Amadori and Giovanni Luca Frassinetti (2016). " paraneoplastic Lipase and amylase production in a patient with small – cell lung cancer: case report " . BMC cancer 16: 118.

22- Kurban S., Akpinar Z., Mehmetoglu I. (2010). "Investigation of serum paraoxonase and arylesterase activities and oxidative stress in patients with multiple Sclerosis". Genel Tip Derg., 20: 13- 7.

23- Nicola Traverso, Roberta Ricciarelli, Maripaola Nitti , Barbara Marengo, Anna Lisa Furfaro, Maria Adelaide Pronzato, Umberto Mario Marinari and Cinzia Domenicotti . (2013). " Role of Glutathione in

cancer progression and chemoresistance ". Oxidative Medicine and Cellular Longevity, Article ID. 972913, 10 pages.

24- Oktay sehker, Burcin Alev-Tuzuner, Sevim Tunali, Esin A. K., Ebru Emekli – Alturfan, Tugba Tunali – Akbay, Leyla Koc-Ozturk, Sule Cetinel , Refiye Yanardag, Aysen Yarat. (2017). "Investigation of the effect of Edaravone on Valproic Acid Induced Tissue Damage in pancreas". Marmara Pharmaceutical Journal, 21/ 3: 570-577.

25- Deveci H.I, Kukurt A., Makav M. and Karapehlivan M. (2017). "Determination of serum paraoxonase activity, total Sialic acid concentration, and oxidative status in cattle with clinical mastitis " Inter J. vet sci. 6(3): 136-146.

26- Mir G.M. et.al. (2013) . " Medicinal applications of copper and its complexes-A Review". World Journal of pharmaceutical research, vol.2 Issue 3, 740-750.

عزل إنزيم التربسين من مصل دم المرضى المصابين بسرطان البنكرياس وقياس بعض المتغيرات

الكيموحيوية

ليلى عبدا لله مصطفى ، زينة احمد محمد

قسم الكيمياء ، كلية العلوم ، جامعة الموصل ، الموصل ، العراق

الملخص

تضمن البحث عزل وتنقية إنزيم التربسين من مصل دم المرضى المصابين بسرطان البنكرياس وتقدير وزنه الجزيئي باستخدام تقنيتي الترشيح الهلامي (باستخدام Sephadex G-50) والهجرة الكهربائية (باستخدام SDS) إذ لوحظ أن الوزن الجزيئي للحزمة البروتينية المفصولة من محلول كبريتات الامونيوم بتسبع (65%) لمصل الدم هو (27000) دالتون. أجريت الدراسة لأربعين مصابا بسرطان البنكرياس تراوحت أعمارهم ما بين (30-70) سنة ممن يراجعون المستشفى الجمهوري ومستشفى الطب الذري في مدينة الموصل, إذ شملت الفحوصات المختبرية قياس مستويات التربسين, كما كلوتاميل ترانسفيريز, امايليز, باراوكسونيز, الكلوتاتايون, حامض السياليك والسيرولوبلازمين. أوضحت نتائج التحليل الإحصائي إن مستوى التربسين والامايليز في مصل الدم قد انخفض بشكل معنوي عند المرضى المصابين بسرطان البنكرياس مقارنة مع مجموعة الأصحاء, في حين إن مستويات كما كلوتاميل ترانسفيريز, باراوكسونيز, الكلوتاتايون, حامض السياليك والسيرولوبلازمين ارتفعت بشكل معنوي عند المرضى المصابين بسرطان البنكرياس مقارنة بالأصحاء.

الكلمات المفتاحية: إنزيم التربسين, سرطان البنكرياس.