IASJ المجلات الاكاديمية العراقية

Tikrit Journal of Pure Science

ISSN: 1813 – 1662 (Print) --- E-ISSN: 2415 – 1726 (Online)

Journal Homepage: http://tjps.tu.edu.iq/index.php/j

Study of some Biochemical Parameters and Partial Purification of amylase from diabetic patients

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https://doi.org/10.25130/tjps.v27i5.14

ARTICLE INFO.

Article history:

-Received: 13 / 1 / 2022

-Accepted: 19 / 7 / 2022

-Available online: / / 2022

Keywords: diabetes, amylase, Parification

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ABSTRACT

The aim of the study was to evaluate the level of amylase in the serum of diabetic patients compared to the level of healthy controls, in glucose, lipids, lipoproteins, thyroid hormones, electrolytes (calcium, potassium, and sodium) and transporter enzymes were also studied. (AST), (ALT),(ALP). the amylase enzyme was purified and the association between the enzyme and the previously mentioned variants was studied. it was studied amylase and other biochemical variables and it was found that there was a significant positive correlation when comparing the enzyme level with the variants (TG, VLDL, ALP) and there was no significant correlation. between enzyme activity and other biochemical variables. Where one peak was obtained in the fourth part of the purification, the degree of purification was 16.1; the result of the enzyme was 108.2% and the specific activity (0.189 ng/ mg, kinetic studies of the partially purified enzyme. The substrat concentration was 10 ng/mg, and the value of Michaelis Menten's constant km (5.55 ng) and maximum velocity Vmax was (0.98 ng/ml) the optimum temperature of the enzyme were reached (37 °C) while the optimum pH was (7.5).

Introduction

The concentration of amylase in the body can be checked in several ways. When the pancreas sometimes gets infections or disorders that may lead to an increase in the amount of the enzyme amylase that it secretes, or a deficiency, so doctors resort to checking the level of amylase in the blood to detect the presence of the pancreas. Diseases or the presence of other disorders in the body that affect the pancreas, and the level of amylase can be checked by a blood or urine test. An elevated concentration of amylase in the body may occur in cases of vomiting, when consuming large amounts of alcohol, in the case of salivary gland infections, or intestinal obstruction[1] . It is an enzyme classified by the pancreas and salivary glands as α-amylases) and there is a small amount of it in the blood ranging between 100-300 IU / L and at this ratio the increase in the blood increases the extraction of this enzyme by the kidneys, and increase the level of the amylase enzyme sometimes during kidney failure, And coma caused by excess sugar and duodenal ulcer lead to pancreatitis. The concentration of amylase in the blood decreases in cases of acute and chronic hepatitis and pancreatic dysfunction, and sometimes during preeclampsia [2]. Amylases are hydrolytic enzymes that break glycoside bonds at the alpha (4-1) sites of starch and glycogen.Amylase is one of the enzymes secreted by the human digestive system. Stimulates the digestion of starchy materials and their conversion into smaller carbohydrate molecules, such as: maltose, which consists of two glucose molecules. The small intestine completes the digestion of starch, and this enzyme is secreted not only by humans, but also by some other organisms, including: plants, bacteria, fungi, and many mammals[3].

Materials and Methods

Buffer 10 mM Tris-HCl pH 7.4

Prepared by dissolving 1.576 g of Tris - HCl in a liter of distilled water and adjusting the pH at 7.4

sephadex G100 Filtration Gel Suspension Solution..

Sodium chloride solution at a concentration of 500 mmol

It was prepared by dissolving 29.25 clouds of NaCl per liter of 0.01M Tris–HCl pH 7.4 solutions.

Procedure Method

1. I used a glass column with a diameter of 1.5 cm and a length of 30 cm and a little glass wool was placed at the end of it to prevent the gel granules from seeping out of the column, then the suspended gel solution was poured into the column slowly and homogeneously to prevent the formation of air bubbles hampering the separation until the height of gel to (12 cm), and then the column was washed with sufficient amounts of 10 M Tris-HCl 7.4 buffer until a flow velocity (1 ml)/min.
2. Add 5 mL of the enzyme after film separation slowly over the surface of the Sephadex G100 gel and leave for 5 minutes to impregnate into the gel column.
3. The separation process was carried out using 100 ml of buffer solution containing 500 mmol of NaCl, collecting 5 ml for each fraction.

Kinetic studies of amylase Kinetic studies of amylase

The kinetics of amylase was studied after it was separated and partially purified from the serum of diabetic patients by gel filtration, including:

Main substance concentration effect

The effect of different concentrations on the activity of the partially purified amylase was studied using different concentrations of it.They are (0.1, 0.05, 0.025, 0.0125, 0.00625, 0.003125, 0.001563) ng / l to find out the effect of the concentration of the substrate on the work of the amylase, and from drawing the relationship between the Velocity and the concentration fellow to the Michal-Menten equation, the values of the Michal-Menten constant were obtained .using the Lenwever-Burke plot[4].

Set the optimum pH

The effect of the pH (for the buffer solution) (10mM Tris - HCl pH 7.4 on the amylase reaction rate) was studied, the same solutions were used different pH (4.5 5.5 6.5 7.5 8.5 9.5) in the presence of the base glucose concentrate 0.05 ng / GTT 37 °C, the relationship between reaction rate and pH was plotted and the optimum pH was determined.

Temperature effect

enzyme, as the reaction was carried out at different temperatures (57, 47, 37, 27, 17, 7) °C in the presence of a buffer solution) 10 mm Tris-HCl) pH 7.4 The base substance concentration was 0.05 ng/L, then the relationship between reaction rate and temperature was plotted with the optimum temperature for the reaction.

Statistical analysis

The results were statistically analyzed using the ANOVA test, the values are represented in the tables (mean ± SD A test was used (t-test (to compare groups, analyze the results of patients and healthy subjects and make a comparison between them), and at the probability level ($p \leq 0.01$) and ($p \leq 0.05$) the graphs were drawn using Excel (2013).

Results and discussion

The results included the statistical values of clinical variables in the blood serum of diabetic patients and in the control group with the methods of work, and the results were as follows Glucose level and lipoprotein level (TG, GLU HDL cholesterol, VLDL, LDL) in diabetic patients and control group.The results are shown in (Table 1-1) below is a significant increase in the concentration level of glucose and low-density lipoprotein LDL at the probability level (p) in diabetic patients compared with the control group. the triglycerides and lipoprotein were very low VLDL and high-density lipoprotein HDL and no significant change was shown when comparing between the group of patients and the control.

Table 1-1: glucose level, lipoprotein level and control group

LDL mg/dl	HDL mg/dl	VLDL mg/dl	TG mg/dl	Cholesterol mg/dl	GUL mg/dl	SE ± Meaning
107.2** 12.1±	47.3 Ns 7.6±	29.6 ns 4.8±	143.7 ns 15.7±	187.0* 5.9±	78.8** 10.5±	the control
147.5 16.6±	43.8 6.2±	29.2 5.4 ±	150.5 11.5±	219.1 11±	214.6 16.1±	The patients

There is a significant statistically significant difference at the level of probability
There is a big difference in the level of probability ($P \leq 0.01$)

There is no moral difference

The higher level of glucose concentration at the probability level (p) of diabetic patients compared to the control group agrees with (Khurshid, 2018 (King MW 2014)[5] if they find that the glucose level in patients is always higher than) 120 mg/ deciliter, researchers also found Amori, R.et.al 2015 [6] an increase in the blood glucose level in patients compared to the control group, and the reason for this high level of glucose is either due to a defect in the production and secretion of insulin, or complete damage to beta cells that Insulin is secreted by the pancreas, or by the receptor cells responsible for glucose consumption. The cause of the defect is due to genetic factors or acquired factors as a result of a deficiency in the level of insulin or its absence, which leads to high blood sugar. And the presence of a significant increase at the probability level (P 0.01) in the level of cholesterol in diabetic patients compared to the control group and agrees with[7] (Yamada, N. et al,1994) the reason for this increased rise in cholesterol is due to the excessive intake of saturated fat and the lack of A calorie-constrained diet in patients with diabetes, as well as due to the analysis

of low-density lipoprotein (LDL) as well as loss of the lipoprotein receptor. B for (LDL-C (contributes to an increase in the level of total cholesterol) Hamdani, RY et al. 2002)[8].

Liver enzymes level (ALT, AST, ALP) in diabetic patients and control group

The results are shown in Table (1-1). There was a significant increase in the level of ALT enzyme activity at the probability level (p) in diabetic patients compared to the control group. There was no significant change in both AST and AL enzymes in the patients group compared to the control group.

Table 1-2: activity level of liver enzymes (AST, ALT, and ALP) and the control group, and the result showed a significant increase in sodium concentration at probability

Na^{+} mg/dl	K^{+} mg/dl	Ca^{2+} mg/dl	ALP U/L	ALT U/L	AST U/L	SE ± Meaning
11.3± 88.9 **	0.8± 4.4 **	0.5±8.8 **	8.9± 59.1 ns	5.7± 21.3 *	1.2±16.8 ns	the control
15.6± 150.7	0.1± 2.7	0.4± 2.4	9.4± 64.9	4.9± 17.3	1.6± 19.3	The patients

There is a significant difference at the level of probability (P≤ 0.05)

There is no moral difference

The increase in the level of ALT enzyme activity in diabetic patients compared to the control group is consistent with A. et al. 2000 (Mahammed, IA et al 1999), [9] (Sultan) [10], the reason for this increase is that liver cells have been destroyed as a result of drugs Ingested by diabetics and also due to bile duct obstruction due to elevated blood lipids, this abnormal elevation also causes structural and functional changes in hepatocytes. It has always been associated with diabetes, and these differences may serve to increase the activity of this enzyme.

While the potassium and calcium concentration showed a significant decrease at the level of probability (p) in diabetic patients compared with the control group.

Table (1-3) electrolyte level (Na^{+}, K^{+}, Ca^{2+}) in diabetic patients and the control group

There is a significant difference at the level of probability (P ≤0.01)

This increase in the level of sodium concentration was at the voltage level (P 0.01) in diabetic patients compared to the control group and this is consistent with 2001 (Richard P. A). While potassium showed a significant decrease at (p≤0.01) in diabetic patients compared to the control group and agrees with (2016) Nabil, AH (et al. Calcium was reabsorbed in renal tubules in case of severe hyperglycemia, and calcium also showed a significant decrease when The probability level (p≤ 0.01) for diabetic patients compared to the control group and agrees with Safaa AR, et al., 2016)). Calcium has a role in regulating blood sugar levels. The hormone insulin helps to do its job by increasing the sensitivity of insulin receptors on the cell surface, and by stimulating the pancreas to secrete insulin. Through its effect on the action of insulin.

Partial purification of amylase from the serum of diabetic patients

Proteins are usually precipitated in the early stages of enzyme purification, by eliminating a large proportion of water and obtaining a degree of purity, and precipitation with salts such as ammonium sulfate is often used for this purpose. 2 (SO_4 (NH_4)) because of its good solubility in water, where the precipitation of salts occurs as a result of the neutralization of protein charges by the action of salt, which leads to a decrease in the solubility and properties of the protein. Sedimentation, and this is called external salting, and this step is one of the important steps adopted by most of the previous studies, as the sedimentation process provides minimizing the size of the enzymatic extract from the largest possible amount of proteins and water. Because it interferes with the activity of the enzyme, it is added in stages to get rid of some protein substances precipitated with the enzyme extract.

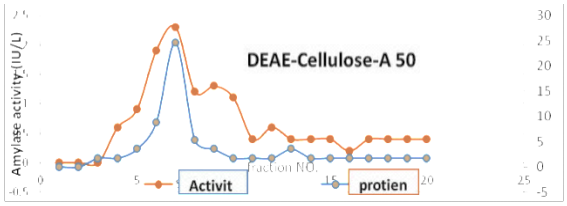

Fig. 1-1: Separation of amylase enzyme from diabetic patients using ion exchange chromatography

Then the isolated enzyme was purified using the gel filtration chromatography method (Sephadex G-100) , as one vertex was obtained, as shown in the figure(1-1) Partially purified with a degree of purification up to 16 Once As shown in the table (1-4).

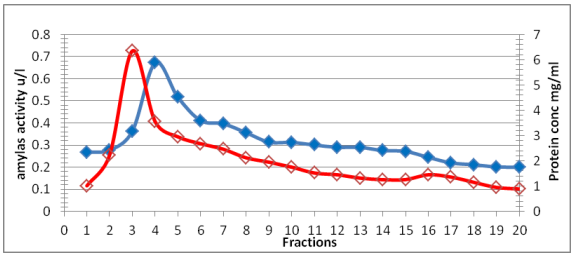

Fig. 1-2: Purification of amylase by gel filtration chromatography

Table 1-4: enzyme separation and purification partially amylase from the serum of diabetic patients

Step	Elute (ml)	Activity (µmol/L)	Total activity U/L	Protein conc. (g/dL)	Total protein (gm)	Specific activity IU/gm	Degree of purification Fold	Yield %
Crud serum	5	0.058	0.29	0.043	0.215	1.349	1	100
Ammonium sulphate	3	0.078	0.234	0.645	1.935	0.121	0.09	80
Dialysis	3	1.052	3.156	0.52	1.56	2.02	1.5	97
(ion exchange) DEAE-Cellulose A50	3	4.561	13.688	0.23	0.69	19.83	14.7	58
Gel filtration Sephadex G – 100	3	11.56	34.68	0.53	0.159	21.8	16.16	77

Partially purified amylase from the serum of diabetic patients

Optimal concentration of CNPG3 and finding Km

The effect of CNPG3 matrix concentration on the rate of the partially purified enzymatic reaction was studied using a Sephadex G-100 column to find the optimal concentration of CNPG3 matrix). From the reaction of the amylase enzyme separated from the blood of diabetic patients, with a high concentration of CNPG3 until the maximum speed is reached at the concentration (10 mM), then the reaction rate begins to stop at high concentrations (the concentration is higher than the optimal concentration from the base level). It is clear from the figure that the enzyme is subject to the Michal-Menten equation, where the resulting graph is a hyperbola (hyperbola), and there are several ways to calculate the value of the Michaelis - MentenKm constant, which is defined as the affinity between the enzyme and the base substance, the higher its value, The affinity between the enzyme and the substrate decreases, and when its value decreases, the affinity between them increases. It is possible to determine the efficiency of enzymatic analogs to catalyze biological reactions and to know the stability of enzymes and the effect of inhibitors and activators on enzyme activity or substrate concentration when the rate of velocity is half the value of the maximum velocity (Vmax), when the enzyme undergoes the micelles-mentin equation, then Km can be calculated for it, Lineweaver-Berke method It is the most accurate and best in practice, due to its ease of use, lack of calculations in it, and its efficiency in demonstrating the accuracy of the experiment calculation, and for the values of the constant K for the gel-filtrated purified enzyme, the above method was as in Figure (1-3) the value of Km amylase equals the enzyme (5.55) Its maximum velocity at the same base material is 0.98 ng/ml. These results contradict some previous studies conducted to determine kinetics. on enzyme supports.

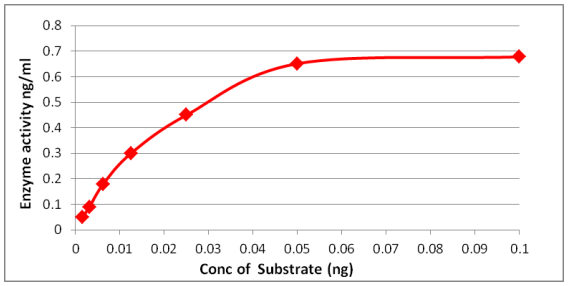

Fig. 1-3: Effect of the concentration of the main substance on the activity of amylase enzyme purified from the serum of diabetic patients using the linear Lenovier-Burke method, value. Km was calculated for the purified enzyme using a Sephadex G-100 column, Figure (1-3) shows the value of Km according to the mentioned method 1 / v vs. 1 / [s]) which was equal to (5.55) mM of the enzyme purified from the serum of diabetic patients.

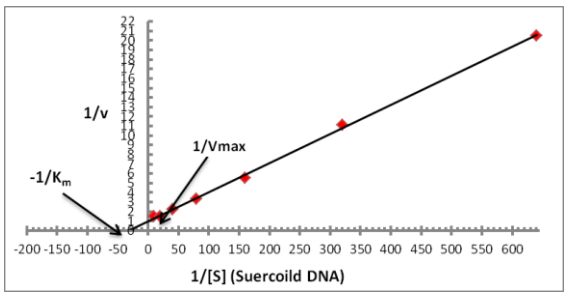

Fig. 1-4: Drawing Lineweaver - pools to calculate Km AndVmax purified amylase enzyme from diabetic patients

The effect of temperature on the reaction rate of the amylase enzyme

Figure (1-4) shows the effect of temperature on the activity of the purified amylase enzyme from the serum of diabetic patients. or slightly higher or lower) than the temperature of the cell containing it, since the rate of the enzymatic reaction increases with increasing temperature until it reaches the optimum temperature for the reaction, after which it begins gradually. The decrease is explained by the effect of high temperatures on the state of ionization of groups on the surface of the enzyme and its basic material, and because enzymes are complex protein molecules whose catalytic activity is affected in the regular triple structure, so high temperatures work to change the enzyme's geometric and natural shape, causing a loss of enzyme activity.

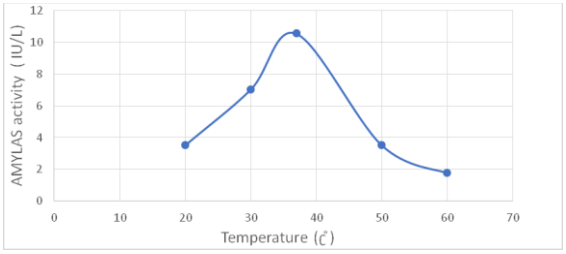

Fig. 1-5: Effect of temperature on the reaction rate of the enzyme purified amylase from diabetic serum

Effect of pH on amylase reaction rate

The degree of pH affects the enzymatic activity as a result of the different nature and chemical composition of the enzyme. The pH is optimal because it is very sensitive to the change in the concentration of hydrogen ion H +, as Figure (1-6) shows that the use of different degrees of pH for the buffer solution (sodium phosphate) used in the enzymatic reaction leads to an increase in the reaction speed with high degrees of pH until maximum speed is reached at the optimum pH which was at pH 7.5 and then enzyme activity decreased at pH above 7.5.

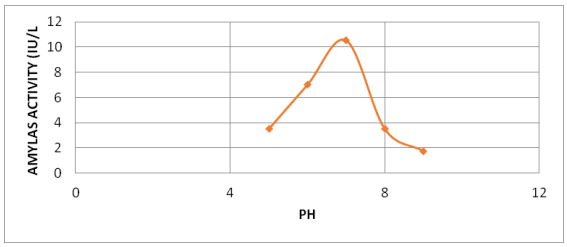

Fig. 1-6: Effect of the reaction rate of the pH enzyme purified from diabetic serum

Conclusion

Through the results that we obtained in this study, High level of amylase enzyme in diabetic patients compared to healthy people. The study showed a significant difference in the level of amylase enzyme when comparing between males and females in the healthy group and the group of patients. It was found that the level of cholesterol, triglycerides and LDL increased VLDL and LDL in diabetic patients, and it was also found that the level of glucose and electrolyte concentrations were increased (Na +, CL - , K +) and enzyme activity (ALT).), concentrations of thyroid hormones (T3, T4) and urea and creatine level in diabetic patients compared with control .The study showed no significant difference in the levels of (HDL, TSH uric acid, AST, AL) in diabetic patients compared to the control group. the enzymatic result was 108.2% and specific activity (0.189 ng/mg).The rate of Km and Vmax for the purified enzyme was (Km = 5.55 ng) and (0.98ng/ml = (Vmax 7- The optimum temperature for enzyme action (37°C) was reached) while the optimum pH was (7.5) .

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دراسة بعض المعاملات البايوكيميائية والتنقية الجزئية للاميليز من مرضى السكري

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الملخص

كان الهدف من الدراسة هو تقييم مستوى الأميليز في مصل مرضى السكري مقارنة بمستوى الضوابط الصحية في الجلوكوز والدهون والبروتينات الدهنية وهرمونات الغدة الدرقية والكهارل (الكالسيوم والبوتاسيوم والصوديوم) وكذلك الإنزيمات الناقلة. درس. تمت تنقية إنزيم الأميليز ودراسة الارتباط بين الإنزيم والمتغيرات المذكورة سابقًا. تم دراسة الأميليز والمتغيرات البيوكيميائية الأخرى. بين نشاط الإنزيم والمتغيرات البيوكيميائية الأخرى. حيث تم الحصول على ذروة واحدة في الجزء الرابع من التطهير، كانت درجة التطهير 16.1 ؛ كانت نتيجة الإنزيم 108.2٪ والنشاط النوعي (0.189 نانوغرام / مجم ، دراسات حركية للإنزيم المنقى جزئياً ، تركيز الركيزة 10 نانوغرام / مجم ، وقيمة ميكايليس مينتين ثابت بالكيلومتر (5.55 نانوغرام) والسرعة القصوى. بلغ (0.98 Vmax نانوغرام / مل) درجة الحرارة المثلى للإنزيم (37 درجة مئوية) بينما كان الرقم الهيدروجيني الأمثل (7.5).