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Isolation and partial Purification and for amylase from diabetic patients and some kinetic properties

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ABSTRACT

Amylase is an enzyme which is secreted from pancreas, and salivary glands (α-Amylase EC3.2.1.1), there is a small amount of this enzyme presents in the blood ranging between 100–300 I.U/L, when this percentage in the blood would increase,it means that the above organs are ill. The study included apartial purification of this enzyme from the blood of diabetic patients using gel, filtration, dialysis, and sephadex G100 gel, the single beak was obtained in the fourth part of purification and the degree of purification reached 16.1 with an enzymatic outcome of 108.2% and specific activity was 0.189ng/mg. The kinetic study of the purified enzyme appeared an optimum concentration of the substrate of 10 ng / mg,the Km was 5.55 ng , maximum acivity Vmax, was 0.98ng / ml,the optimum temp. was (37C°) and the opt. pH was 7.5.

Introduction

Amylase is an enzyme secreted, from the pancreas and salivary glands (α-Amylases EC3.2.1.1), and there is a small amount of it in the blood ranging between 100 - 300 IU /L, and when this concentration increases in the blood, this enzyme extract increases through the kidneys. The level of amylase was rises kidney failure, hypoglycemia and, duodenal ulcers leading to pancreatitis disease, and decreases in cases of acute and chronic liver infections, weakening of the pancreas, and sometimes during preeclampsia[1]. The optimum pH and temperature for amylase were 6.9-7.0 [2] and 37 °,40 ° C, respectively and this enzyme activity continues until (50 °C). There are three types of alpha-amylase and beta -Amylase and Gamma-Amylase, and this enzyme is found in the salivary gland and the pancreas[3,4,5], this glands secreted amylase to hydrolyzed starch into oligosaccharides and to glucose with α(1-6) glucosidase to supply the body with energy**[4-7]**. Amylase is one of the important enzymes that have clinical importance in the diagnosis of many diseases, including acute pancreatitis and blockage of the pancreas ducts, since one of the causes of pancreatitis is the formation of stones in the ,gallbladder[1,8,9,10], as studies have proven high The activity of alpha-amylase in the serum of people with insulin-dependent diabetes[5,11,12,13,14,15].

Methods

Amylase was purified from the serum of. diabetic patients (5 mL of serum) using the following steps : 1-addition ammonium sulphate (80%, 4g, The precipitate was dissolved in a less amount of phosphate buffer solution pH 7.4); 2-Dialysis, Tris-Hcl pH 7.4; 3-Gel filtration Chromatography (Sephadex G100, pH 7.4 flow rate was (2 ml /min) column (30x1.5 cm). The kinetic study was done according to the literature, 4-Ion exchange: diethyl amino ethyl cellulose A-50 (DEAE) (column (20x2 cm); concentrations of sodium chloride solution, (0.1-0.4)M; The flow rate was (2ml/4 min)[16].

For used solutions

1. Buffer solution 10mM Tris - HCl pH 7.4

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

2. Sephadex G100 gel filtration suspension

It was prepared by dissolving 2.5 gm of Sephadex G100 column filler in 200 ml of 10mM Tris - HCl pH 7.4 buffer and left the solution for (24-20) hours at 4 ° C, during which time the buffer was changed several times to remove the fine particles from the solution because Their presence reduces the velocity of the leachate flow through the column.

3. Sodium chloride solution at a concentration of 500 mmol

Prepared by dissolving 29.25 g of NaCl in a liter of 0.01M Tris-HCl pH 7.4 buffer.

Results and discussion

Proteins are usually precipitated in the first stages of enzyme purification by removing a large percentage of water and obtaining a degree of purity. Often, salt precipitate such as ammonium sulfate are used for ,this purpose because of their good solubility in water. this step is called Salting out and its one of the important steps adopted by most of the previous studies, as the, sedimentation process provides a decrease in the volume of the enzymatic extract[17] .
Purification of amylase was summarized in table (1). It was purified in several steps ; the yield was 37.52% and specific activity was (3.98IU/mg). Dialysis was done by Tris-HCl buffer pH 7.4 to obtain 1.08 fold of purification, the yield was 45.03% and specific activity was (4.05 IU/mg). Gel filtration ,Sephadex G100 gave a single band of purified enzyme; with 16.1 folds of purification, yield percentage was 67.5% and specific activity was (59.54 IU/mg) as shown in fig (1). Ion exchange chromatography was used to obtain 2.863 fold of purification and the yield was 5253% and specific activity was (10.68 IU/mg) as shown in fig (2).

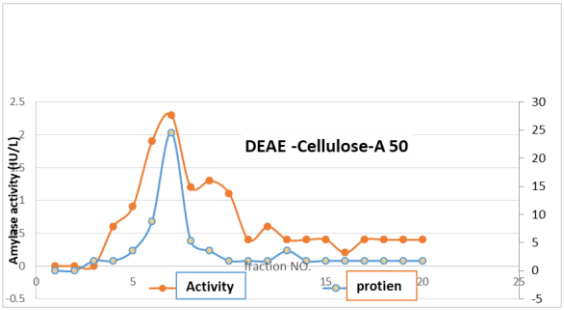

Fig. 1: purification of amylase using Ion exchange

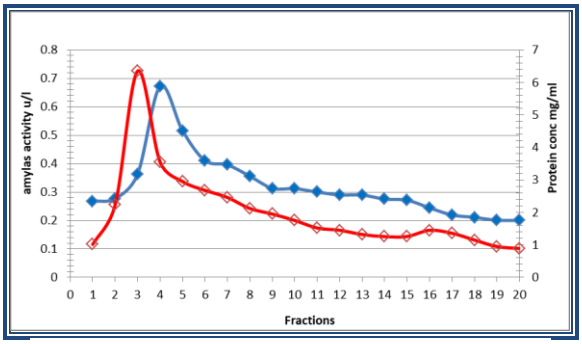

Fig. 2: purification of amylase using Gel filtaration

Table 1: Partial purification of Amylase, in serum of diabetic patients using ammonium sulphate, gel filtration and Ion exchange chromatography

Step	Elute (ml)	Activity (U/L)	Total activity U/L	Protein conc. (gm/dL)	Total protein (gm)	Specific activity (I U/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	.234*0*	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

Kinetic study of partial purified amylase :

The optimal concentration for the substrate

The effect of the concentration of the 2-chloro-4-nitrophenol (CNPG3) substrate was studied on the activity of the partially purified enzymatic reaction using the Sephadex G-100 column, and finding the optimal concentration of the substrate CNPG3, as shown in fig (3) shows an increase in the activity of the amylase separated from the diabetic patients. when substrate concentration increases the, activity of the enzyme was increased until the maximum activity (maximum concentration (10 mM)), after which the activity begins to stop at high concentrations (the higher concentration than the optimal concentration of the substrate). It is clear from Figure (3) that the enzyme is followed to the Michaels – Mentens equation where the resulting graphical form is hyperbolic, and there are several methods for calculating the, value of the Michaels – Mentens constant, Km, which is defined as the affinity between the enzyme and the substrate, the higher the value, the lower the affinity. Between the enzyme and the substrate, and when its value decreases, the affenfy between them increases[18]. or the concentration of the substrate when the activity is half the value of the maximum velocity (Vmax)[19]**,** the method of Lineweaver,-Burke plot was used to calculate the values of the km constant and Vmax of the purified enzyme Fig.(4), and the km value of the amylase
was equal to (0.0333) mmol as well as its maximum velocity equal to (0.98ng/ml), and these results varied with some studies The previous one was conducted to determine the enzyme kinematic constants and the differences between all these studies were clear and almost natural due to the different sources from which the enzyme was purified and the different ,methods used in purification.

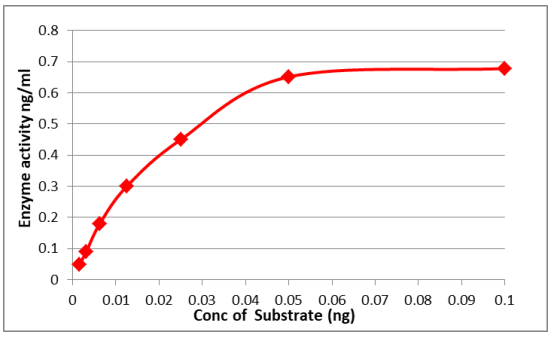

Fig. 3: effect of substrate concentration on amylase activity

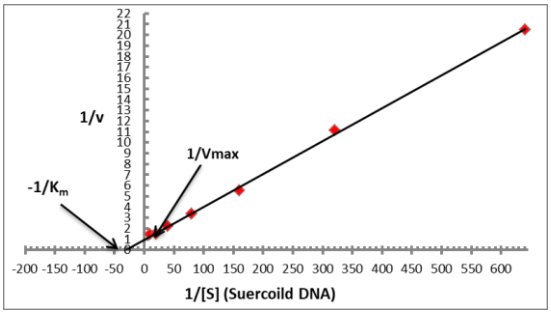

Fig. 4: Lineweaver-Burke plot

The effect of temperature on the activity of amylase

Fig. (5) shows the effect of temperature on the activity of the purified amylase from diabetic serum, as the, results showed an increase in the activity of enzyme with increasing temperature, and the maximum temperature was 37C°, and the enzymes usually have an optimal temperature (equal or higher Or slightly less) than the temperature of the cell that contains it, the activity of the enzymatic reaction increases with increasing temperatures, until the optimal degree of the reaction after it begins to decrease gradually due to the denaturation or damage of the enzyme molecule, as this reduces the enzyme activity this explains the decrease Through an effect High temperatures to the ionization state of the groups present on the surface of the enzyme and its base material, and the fact that the enzymes are complex protein molecules whose catalytic activity is affected in the regular, tripartite structural composition, so high temperatures work to change the geometric and natural form of the enzyme, causing the enzyme to lose its activity[20] .

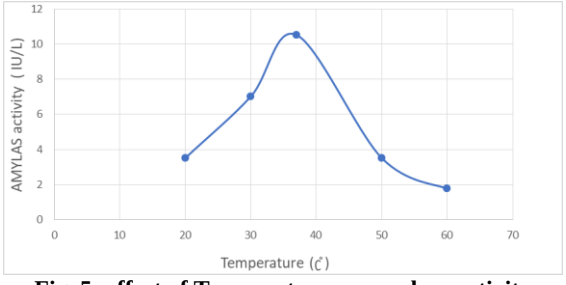

Fig. 5: effect of Temperature on amylase activity

The effect of pH on the activity of amylase

The degree of pH affects the enzymatic activity due to the different nature of the enzyme and its chemical composition, as well as the presence of multiple ion groups that the enzyme carries, as, the enzymes operate at an optimal pH degree because they are very sensitive to change with the concentration of hydrogen ion H+ [21], as shown in Fig. (6) That the use of different degrees of pH of the buffer solution (sodium phosphate) used in the enzymatic reaction causes an increase in the reaction velocity with a high pH score until ,the maximum velocity is reached at the optimal pH that was at 7.5 pH then the enzyme activity decreases at pH levels higher than 7.5.

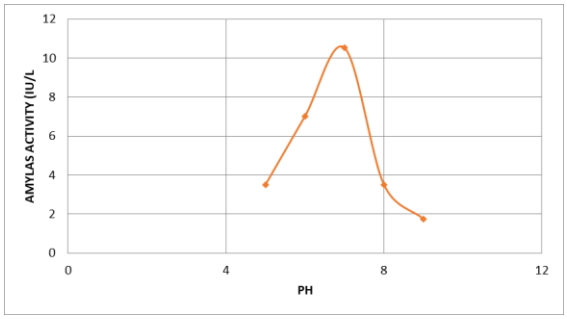

Fig. 6: effect of pH on amylase activity

The levels of amylase, in the case of chronic pancreatitis: chronic pancreatitis) is less than it is in the case of acute pancreatitis for a period ranging between 8-72 hours and the concentration of amylase reaches the highest height within 24 to 30 hours from the beginning of feeling sick, where its levels reach 550 units and sometimes 2000 units, And the rise continues for several days, then the level decreases and returns naturally in a period ,that does not exceed three days, and this is due to the ability of the enzyme to leak through the kidney to the diuresis, and for this reason, the blood samples used to measure the activity of amylase must be collected from the patient as soon as possible in order to have a personal value They can be relied upon, and taken into consideration[22].

Aim of the study

Study the level of amylase enzyme in diabetic patients and compare it with apparently healthy subjects as a control group1.

Purification of the amylase enzyme, disease, disease, disease, disease, disease, disease, computed tomography, column and weight estimation, approximate to it2.

Study the kinetics of the enzyme, such as temperature, acidity function, and base material concentration, as well as calculating the micelles constant of km of the enzyme and the maximum velocity Vmax3.

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تنقية جزئية لأنزيم الاميليز من مصل مرضى السكري

وسن عبد الحسن , فراس طاهر ماهر

قسم الكيمياء ، كلية العلوم , جامعة تكريت , تكريت ,العراق

الملخص

الاميليز هو إنزيم يُفرز من البنكرياس والغدد اللعابية (α-Amylases EC3.2.1.1)، وتوجد كمية بسيطة منه بالدم تتراوح ما بين 100 – 300 وحدة دولية / لتر، وعند ازدياد هذه النسبة في الدم يزداد استخراج هذا الإنزيم عن طريق الكلى.

تم جمع العينات من مستشفى صلاح الدين في تكريت ، وتم قياس التحاليل في مختبر مستشفى صلاح الدين والذين تمت معاينتُهم من قبل الطبيب المختص.

حيث شملت الدراسة تنقية انزيم الاميليز جزئياً ومن مصل مرضى داء السكري وذلك باستعمال تقنية الترشيح الهلامي والديلزة وباستعمال هلام سيفادكس G100, اذ تم الحصول على قمة واحدة في الجزء الرابع من اجزاء التنقية وبلغت درجة التنقية 16.1 بحصيلة انزيمية 108.2% وفعالية نوعية (0.189ng/mg) واجريت الدراسات الحركية للأنزيم المنقى جزئياً، فكان التركيزو الامثل للمادة الاساس ((10 ng/mg), وبلغت قيمة ثابت ميكالس– منتن Km (5.55 ng) والسرعة القصوى Vmax (0.98ng/ml), اما درجة الحرارة المثلى لعمل الانزيم فبلغت (37C°) في حين كان الاس الهيدروجيني pH الامثل هو(7.5).