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The Study of Serum GOT(Glutamic Oxalacetic Transaminase) Activity and Some kinetic Parameters in Patient with Pulmonary Tuberculosis

P. A.Ismahil , L. M.Hassan
Department of Chemistry ,College of Education Science,University of Salahaddin

Abstract

In the present study, serum GOT(Glutamic Oxalacetic Transaminase) was purified, the purified enzyme showed the maximum activity at 37ºC and pH 7.5. During purification of serum GOT ion exchange chromatography lead to four separate forms (termed I, II, III and IV). GOT II with the highest specific activity was pure after chromatography on Sephacryl S-300. . S.GOT levels were investigated in serum samples from patient with pulmonary tuberculosis .The S.GOT levels were determined at and 37ć .The study revealed that the serum GOT activity was higher in patient of tuberculous pulmonary than in control subjects.The mean serum GOT activity in the patients group was(90±8 IU/L) as compared to control group (27±0.65IU/L) showing a highly significant ($p<0.001$)difference .The study was concentrated to comprehensive measurement of the rate reaction kinetics(Km,Vmax,Hill coefficient),the first order forward rate constant k_1 and half life $t_{1/2}$ of the enzyme reaction in both normal and tuberculosis serum to evaluate any change In the number and structure of the active site then the rate of enzyme –substrate binding.

Key words :Glutamic Oxalacetic Transaminase.Tuberculosis

Introduction

Tuberculosis is a chronic bacterial infection caused by Mycobacterium tuberculosis and characterized by the formation of granulomas in infected tissue and by cell –mediated hypersensitivity. The usual site of diseases is the lung ,but other organs may be involved [1,2,3],Tuberculosis has emerged as one of the most lethal diseases man has sever faced in short span of time become a major health problem in the third word. The severity of the diseases can be judged by the fact that it affects all ages [4,5,6].Mycobacterium tuberculosis is responsible for more morbidity in humans than any other bacterial diseases.Mycobacterium infects 1.1 billion people per year which is equal to 33% of the entire word population [7,8,9] Glutamic oxalacetic transaminase is a tissue enzyme that catalyzes the transfer of amino and keto group between alpha-amino acid and alpha-keto acid .The GOT exist in the tissues of many organs .Necrotic activity in these organs causes a release of abnormal quantities of enzyme into the blood .An increase in serum GOT activity has been described in several diseases as myocardial infarction. GOT activity is also increased with various types of liver diseases like viral hepatitis or in liver damage and some time with renal diseases .AST or necrosis and inflammation of heart,liver and muscle cells [10,11,12,13]

Experimental

*Samples collection and Separation

Blood analysis is usually done on venous of capillary blood.60 samples of the blood were through out the investigation of GOT enzyme activity ,35 of these samples were of

tuberculosis ,while 25 of these samples were of healthy individuals .5 ml of blood has been collected and allowed standing at room temperature until it has clotted.

Restriction of the clot may be assisted by gentle loosing it from the walls of the container. The serum was separated by centrifugation at 3000 r.p.m for 15 minutes.Separated serum was used on the same day of determination

Chemicals

All chemicals used were of high analysis grade and parched from BDH ,FLUKA

***Instruments and Apparatus**

-Sepctrophotometer, supertonic 601
-Philips pH-meter
-Thermostatic water bath
-Centrifuge T.5

*Methods

A-Estimation of S.GOT activity

S.GOT activity was determined by measurement of oxaloacetate produced during the assay according to the method of Bergmeyer and Bernt (1965). The samples were incubated at 37◦C for 1 h in tubes containing 100 µM aspartic acid, 2µM α-ketoglutaric acid, 20mM sodium phosphate buffer, pH 7.5 and appropriate amount of enzyme to give a final volume of 1.0ml.

The reaction was stopped by the addition of 1ml 2,4-dinitrophenylhydrazine reagent and allowed to incubate 20min at room temperature and 10ml 0.4N sodium hydroxide was added.

The absorbance was recorded at 505 nm. The enzymatically liberated oxaloacetate was calculated from a standard curve absorbed at 510 nm ,using series concentration of substrate[S] Fig(3).

Purification of aspartate transaminase

Step 1: Freshly drawn, heparinized human blood is centrifuged at 5000 rpm for 30 min, and the plasma is treated with ammonium sulfate. The protein fraction precipitating between 30 and 80% saturation is collected

Step 2: The pooled supernatants (630ml) were placed in a 60°C water bath for 40min with frequent mixing and then centrifuged at 100OOg for 30min.

Step 3: The supernatant from stage 2 was further purified by (NH4)2SO4 precipitation. Optimum precipitation occurred between 64 and 72% saturation, with an overall purification factor of 69 and a yield of 37%. and a yield of 30% were obtained. Protein precipitating overnight in the optimum range was collected and redissolved in 0.01 M-sodium phosphate buffer, pH7.4, dialyzed against the same buffer

Step 4:**DEAE-cellulose chromatography**

The dialyzed supernatant from Step 3 was applied directly to a DEAEcellulose column (20×2.6 cm i.d.) equilibrated with 50mM sodium phosphate buffer, pH 7.5. The adsorbed material was eluted with a stepwise gradient ranging from 0 to 0.4M NaCl prepared in the same buffer at a flow rate of 60ml/h and 10ml fractions were collected. Protein fractions exhibitingAST activity were eluted with 0.0, 0.05, 0.1 and 0.2M NaCl, respectively and designated AST I, II, III and IV, according to elution order.

Step 5: **Sephacryl chromatography**

AST II was applied to a Sephacryl S-300 column (95×1.6 cm i.d.) equilibrated with 50mM sodium phosphate buffer, pH 7.5 and developed at a flow rate of 60ml/h and 3ml fractions were collected. The AST II was eluted withthe same buffer.

Protein determination

Protein was determined either by measuring the absorbance at 280 nm (Warburg and Christian, 1942) or by the method of Bradford (1976) using bovine serum albumin as a standard

B-Estimation of physical constant (Km& Vmax) values

The values of Km &Vmax was determined in normal and tuberculosis cases the same experimental protocol of activity was applied and the only difference is the use of different substrate concentration .The data obtained were examined for Km & Vmax by using different methods of plotting data .Theses were of

1-Michaeles-Mentin plot in which the rate of reaction(V)is plotted Vs [S],directly

2-Lineweaver-Burk plot a reciprocal plot of 1/V Vs 1/[S] has been applied

C-Estimation of the rate association constant (K_1),half-life time ($t_{1/2}$)

Following time course reaction ,the order of the enzymatic reaction (K_1) and half life times($t_{1/2}$) were determined .The same protocol of the activity was applied at constant [S] but the time of incubation was varied (fig5,6)

D-Estimation of the activation Energies(Ea*)

The same protocol of GOT determination was adopted and the only difference is the use of various incubation temperature (fig7)

Results and Discussion

The purification of AST is summarized in Table 1. From the elution profile of the chromatography on DEAE-cellulose (Figure 1a), it can be seen that AST activity was detected in four peaks: the negative adsorbed fractions and the fractions eluted with 0.05, 0.1 and 0.2M sodium chloride and designated as GOT I, II, III and IV, respectively. Further purification was restricted to GOT II. A Sephacryl S-300 column (Figure1b) was used to obtain GOT II. with the highest possible specific activity (3.63 units/mg protein).

The activity of GOT in tuberculosis was estimated quantitatively and compared with that of normal using developed calorimetric method .Data obtained revealed specific elevation of GOT activity (90±8.1 IU/L) in case of tuberculosis fig(2) comparing with normal individuals (27±0.65 IU/L) .This is due to that serum GOT level positively corrected to the diseases extent in tuberculosis of immunocomptenl patients .The presence of elevated enzyme activity in the plasma may indicate tissue damage accompanied by increased release of intracellular enzymes. Many diseases that cause tissue damage result in an increased release of intracellular enzyme in to the plasma .The level of specific enzyme activity in the plasma frequently correlates with the extent of tissue damage. Thus ,the degree of elevation of a particular enzyme activity in plasma is often useful in evaluating the prognosis of the patient [15].The Km values for the serum GOT decreased in tuberculosis patient(0.64 ±0.5mM)fig.4 table2) as compared to normal group(1.03 ±0.8mM) .This means that the affinity of the enzyme for their substrate was affected by the diseases and chemical structure (ionic state) of the active sites become more suitable for the substrate for binding [16].While the Vmax for the serum GOT increased in tuberculosis patients (90±8 IU/L) (IU/L) (fig 3 table2) as compared to normal(27±0.65 IU/L) these results are acceptable because there is an increase in lymphocytes and macrophages activities and concentration in tuberculosis patient which affect the rate of general metabolism [17].So increasing GOT activities and concentration (number of active site).(Fig 5 table 3)indicates that the GOT enzyme reaction in both normal and tuberculosis patients reaction are of the pseudo first order type from these figures, the forward reaction rate constant(association rate constant)(K_1) and half time ($t_{1/2}$) were estimated in both cases using the following equation

The results showed that the (K_1) for serum GOT increased in tuberculosis patient but ($t_{1/2}$) decreased as compared with normal group(fig 6 table3), so one can conclude that the diseases may affect equilibrium constant(K_{eq}) of (Ea*)complex formation through out increasing the association constant [18,19].

The results also showed that the tuberculosis affected (decreased) the half life time of serum GOT enzyme reaction . This may be due to the acceleration of their activities by increasing the GOT concentration and the affinity of the GOT to their substrate .The hill coefficient(n) value of the serum enzyme binding site to their substrate was estimated from hill plot fig6.The results (table3) revealed that there were no cooperation and no significant change in (n)value in the case of tuberculosis comparing with that of normal groups.(Fig7 table4) represent the Arrhenius plot for the serum GOT enzyme in normal and tuberculosis patient from the figures the energy of activation of the enzyme substrate reaction were estimated (table4).The results show that (Ea*)for enzyme reaction decreases in tuberculosis case .These results indicate that the diseases may affect the mechanism of the enzyme reactions, this predicts that there is another pathway for the enzyme-substrate complex((Ea*)[20]

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Table (1): Purification scheme for serum GOT II

Purification step	Total units	Total protein mg	Specific activity(units/ mg) protein	Fold purification	Recovery%
DEAE-cellulose 0.01 M NaCl (I)	0.4	10	0.4	3.3	7.8
0.05 M NaCl (II)	16.4	26	0.63	5.25	32.1
0.1 M NaCl (III)	11.5	46	0.25	2.1	22.5
0.2 M NaCl (IV)	13	75	0.173	1.44	25.5
Sephacryl S-300 AST II	13.8	3.8	3.63	30.25	27

∗One unit of GOT activity was defined as the amount of enzyme producing 1 µmol oxaloacetate (pyruvate) per h under standard assay conditions.

Table(2):Km and Vmax value of Normal& Tuberculosis S GOT

Enzyme	Tuberculosis		Normal	
GOT	Km(mM)	Vmax(IU/L)	Km(mM)	Vmax(IU/L)
	0.64	90	1.03	27

Table(3):Enzyme reaction parameters of Normal and Tuberculosis SGOT

Enzyme	K_1(min-¹)		t1/2		Hil Coefficient (n)	
	Normal	Tuberculosis	Normal	Tuberculosis	Normal	Tuberculosis
GOT♀	0.028	0.045	24.75	15.4	1.09	1.9 7

Table(4):Activation energy of Normal and Tuberculosis S GOT

Enzyme	Activation energy Ea	
	Normal	Tuberculosis
GOT	8882.18	7925.18

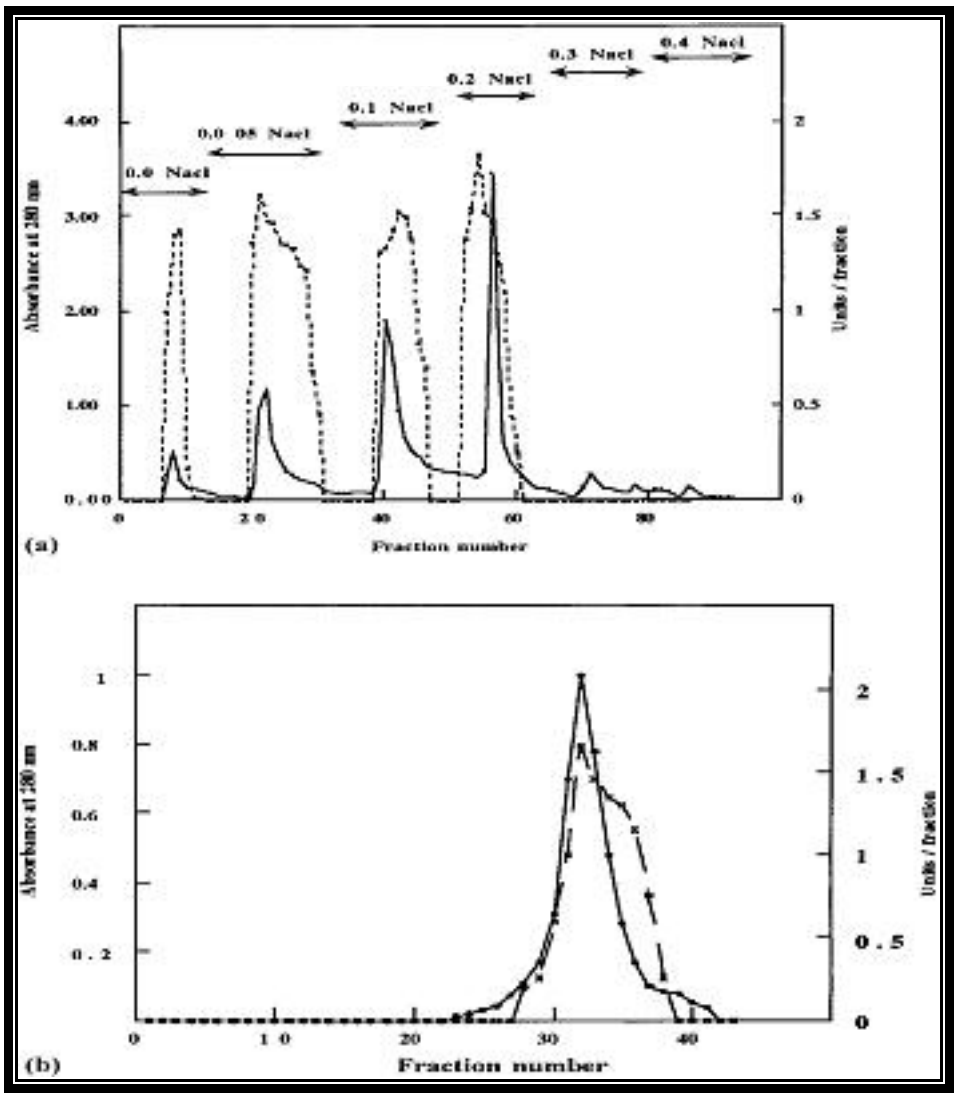
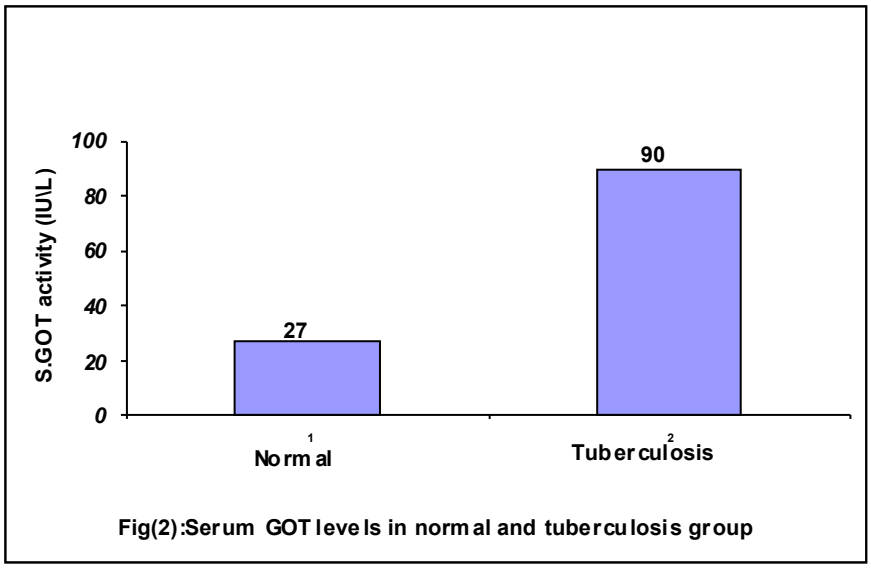
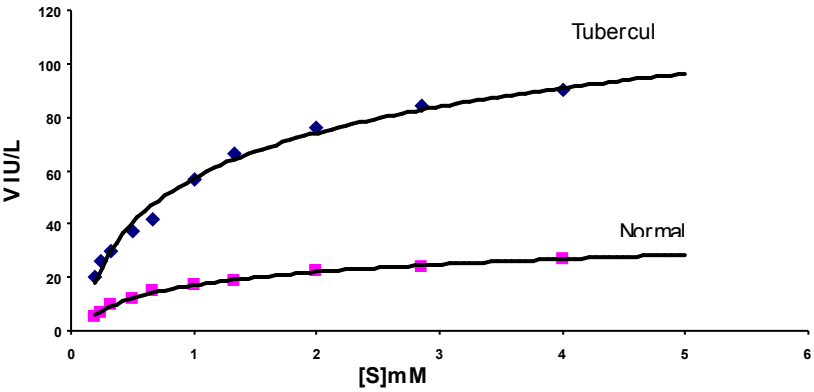


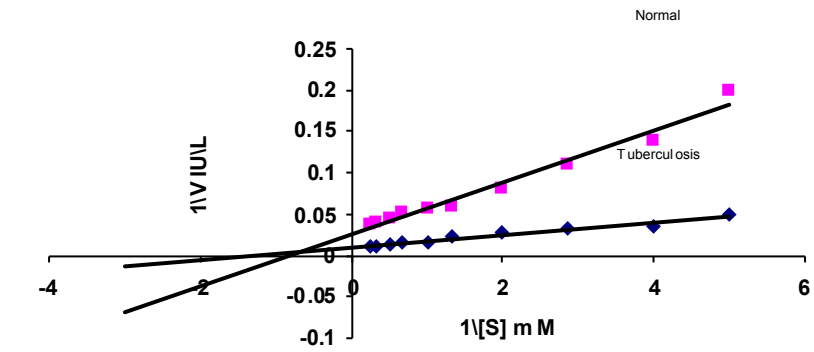
Fig. (1) (a): A typical elution profile for the chromatography of. Serum GOT on DEAE-cellulose column (20×2.6 cm i.d.) previously equilibrated with 20mM sodium phosphate buffer, pH 7.5 at a flow rate of 60 ml/h and 10ml fractions. (b) A typical elution profile for the chromatography of Serum GOT II DEAE-cellulose fraction on Sephacryl S-300 column (95×1.6 cm i.d.) previously equilibrated with 20mM sodium phosphate buffer, pH 7.5 at a flow rate of 60ml/h and 3 ml fractions. Absorbance at 280 nm (•—•)GOT ,activity



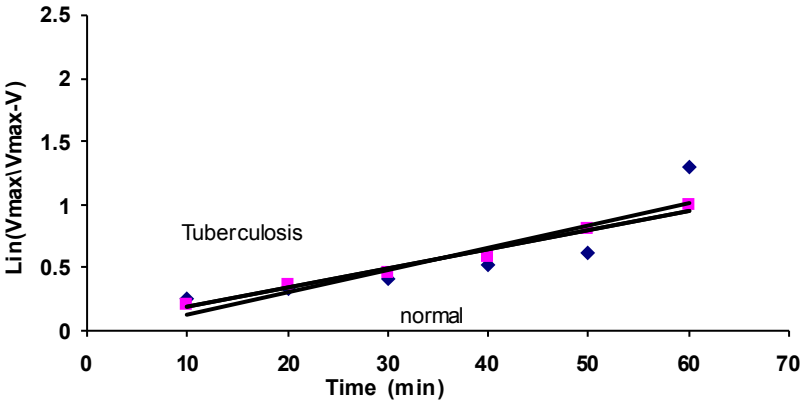
Fig(2):Serum GOT levels in normal and tuberculosis group



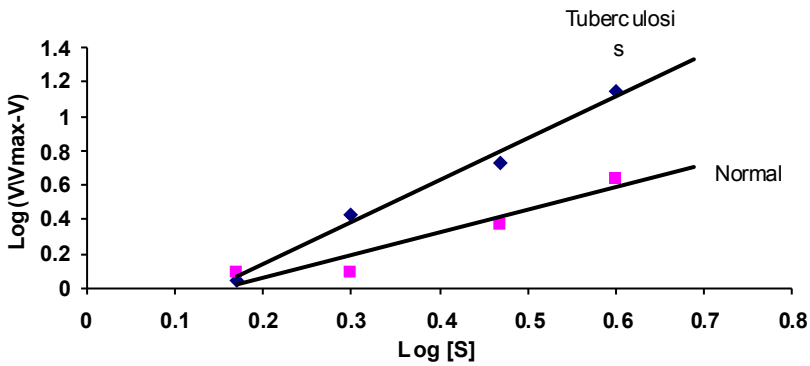
Fig(3):Michaelis-Menten plot for both normal and tuberculosis



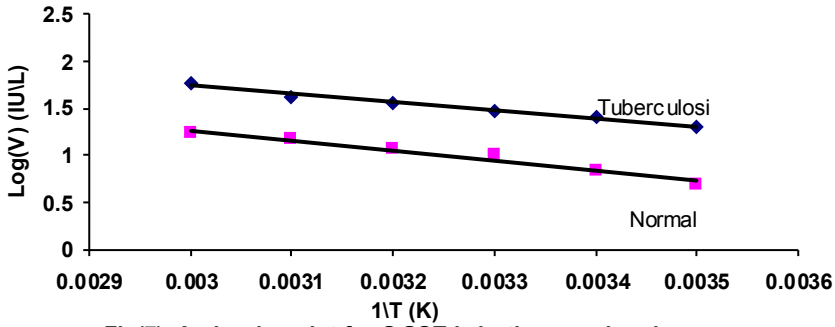
Fig(4): Linweaver-Burk plot S.GOT for both tuberculosis and normal serum



Fif(5):pseudo first order plot for S.GOT in both normal and tuberculosis



Fig(6):Hill plot for S.GOT in both normal and Tuberculosis



Fig(7): Arrhenius plot for S.GOT in both normal and tuberculosis

دراسة نشاط الانزيم (GOT) و بعض الدوال الحركية فى مصول المرضى المصابين بالتدرن الرئوى

بروين عبد الصمد اسماعيل، لطفية محمد حسن
قسم الكيمياء ،كلية التربية/الاقسام العلمية، جامعة صلاح الدين
قسم الكيمياء ،كلية التربية/الاقسام العلمية،جامعة صلاح لدين

الخلاصة

فى الدراسة الحالية تم تنقية الانزيم GOT فى المصل اذ ابدى الانزيم المنقى اعظم فعالية فى حرارة37 و اس الهيدروجينى 7.4 درجة و خلا ل عملية التنقية ادى كروموتوغرافيا التبادل الا يونى الى فصل الانزيم الى اربعة اشكال. و قد بحثت مستويات الانزيم GOT فى مصول المرضى المصابين بالتدرن الرئوى و اظهرت الدراسة
ن نشاط الانزيم فى مصول المرضى اعلى من معدلا ته فى المصول الطبيعية اذ ان معدل نشاط الانزيم فى مجموعة مرضى التدرن الرئوى بلغت (90±8 IU/L IU/L)) مقارنة بالمجموعة الطبيعية والبالغة(27±0.65IU/L) وبينت ارتفاعا معنويا (P<0.001 كما ابدى الانزيم GOT قمة نشاطه فى اس الهيدروجينى 7.4 ودرجة الحرارة 37ć.ولقد ركزت الدراسة على متابعة ميكانيكية التفاعل الانزيمى فى كلا النمواجين (الطبيعى و التدرن الرئوى) ،اذ شملت دراسة(قيم Km,Vmax ،معدلات ثابت التكوين (K_1) وزمن العمر النصفى)$(t_{1/2/})$(لتقيم اى تغير حاصل فى عدد وتركيب المواقع النشطة الموجودة على الانزيم ومن ثم تغير معدل سرعة التفاعل الانزيمى