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A kinetic study of the rate reaction of gamma-glutamyltransferase in hepatitis patients

KEYWORDS: GGT enzyme,hepatitis,thermodynamic parameters

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ABSTRACT *Serum GGT activity has been investigated in hepatitis, activities were found elevated in this case studied at basic pH and 37 C0.The study was concentrated to comprehensive determination of the rate reaction kinetics of the enzyme reaction in both normal and hepatitis sera. The pseudo first –order plot reflects both values of the first-order association constant K1 and the half life time (t1/2) of the enzymatic reaction. The activation energy of the reaction (ES-complex formation) with Hill coefficient (n) were both estimated using Arrhenius and Hill plots respectively. The activation energy E* for GGT reaction decreased in hepatitis patients comparing with normal group ,and the (ΔH* and ΔG*) values for GGT enzyme in normal and hepatitis were positive, while ΔS* value for GGT enzyme in normal and hepatitis were negative. These data showed that in hepatitis patients there was a change in the mechanism of the ES- complex formation in the GGT enzyme reactions.*

Introduction:
Hepatitis is a medical condition defined by the inflammation of the liver and characterized by the presence of inflammatory cells in the tissue of the liver organ(1).Gamma-glutamyltransferase (GGT) catalyzes the transfer of the gamma-glutamyl moiety of glutathione to an acceptor that may be an amino acid,apeptide or water forming glutamate(2). GGT found in many tissue ,the most notable one being the liver ,and has significance in medicine as a diagnostic marker. Elevation of S.GGT activity is frequently interpreted as an index of hepatobiliary dysfunction and as a nonspecific marker of excessive alcohol use (3). Enzymes represent amajor components of the biological fluids. They are maintained life continuity throughout their catalysis reactions.

Thermodynamic data on enzyme-catalyzed reactions play an important role in the prediction of the extent of the reaction and the position of equilibrium for any process in which these reaction occur. The importance of understanding the thermodynamic of these biochemical reactions was emphasized by Krebs and Kornberg (4). Their monograph also contains a useful appendix on Gibbs energy data of biological interest and a table on the thermodynamics of enzyme-catalyzed reactions.

In enzyme reaction, activation of the substrate occurs by the formation of the complex enzyme-substrate (ES) and the process has much in common with the formation of the activation complex of the absolute rate theory (5). The amount of data available at that time was extremely limited. Reviews on various aspects of this subject have subsequently appeared (6,7,8). Each of these reviews, however , has been limited in the extent of coverage given to this area and no comprehensive review exists.

In order to obtain cooperative binding of substrate ,the enzyme must clearly have more than one binding site, a number of models were adopted to cooperative binding, one of these models is that considered by a Hill in 1949, in which Hill coefficient (n) would correspond to the number of binding sites and equal to one so that a reaction would describe by a rectangular hyperbola(5).

In this study, the author has been carried out a typical determination of the rate reaction kinetics in normal and hepatitis for their reaction catalyzes by GGT enzyme.

Material and methods:
A-Chemicals:
These were chosen for specific GGT activity determination and all other experiments related, they were of analytical grade, and highly purified, purchased from BDH and FLUKA.

B-blood collection and serum separation:-
These were of 30 normal healthy adults in addition to 50 samples represents hepatitis individuals ,aged between 21-67 years, from where blood samples were collected, these samples were diagnosed by consultants and proved by GGT test (hepatitis),they are no accompanied diseases, the blood left at room temperature until it has clotted. After clot formation the serum isolated by centrifugation, the separated serum was used on the same day of enzyme activity.

C-Estimation of GGT activity:
The GGT activity was determined by the hydrolysis of g-glutamyl p-nitroanilide in the presence of the acceptor (9). Standard assay included final reagent concentration 2mM of gamma-glutamyl p-nitroanilide, 62 mM of glycylglycine and 95 mM of Tris-HCl, pH 8.1. The rate of p-nitroanilide formation was measured at 405 nm by using spectrophotometer. The results were expressed as U/L ,one unit of enzyme represents the amount of enzyme that catalyzes the release of 1mmol of nitroaniline/min.

GGT activity was calculated using the equation:

GGT activity (IU/L) = (Δ Abs /min) x 2121

Where the absorbance read at 405 nm .

D-Time of incubation effect on GGT activity:
Experiment such that in (estimation of GGT activity) ,has also been adopted in this experiment, the only difference is the various time of incubation of 10,30,60,90, and 120 min. have been applied.

E-Determination of GGT reaction orders, rate constant K1 ,half life time (t1/2) and Hill coefficient(n):
Using time course the order of the enzyme reactions, rate of constant of the forward reactions K1, and the half life time (t1/2) were determined. Figures obtained by plotting Ln(Vmax /Vmax-V) vs time (t) in minutes. A straight line indicated a first order reaction,from the plots the (K1) values were determined (10),and using the following equation,(t1/2) values were calculated:

$t1/2=Ln2/K_1=0.693/K_1$

the Hill coefficient (n) were determined from Hill plots by

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drawing figures between Log(V/Vmax-V) vs Log [s] and Hill coeffeicient (n) values were calculated by Log(V/Vmax-V)= n Log[s]-LogK1(11,12).

F- Determination of activation energy (E*),ΔH*,ΔG* and ΔS*:

The same protocol for GGT activity determination was adopted and the only difference is the use of various temperature we calculate all thermodynamic parameters of transition state(13,14,15).

Results:

Follow up the activities of GGT in the 50 hepatitis samples,it was seen that there was an elevation occurred in hepatitis(81.19 ± 1. 24 U/L) when compared with normal activity (17.31 ± 0.67 U/L) as showed in (table 1).

Group	GGT activity (Mean±SD) IU/L
Normal	17.31±0.67
Hepatitis	81.19±1.24

Table (1): Levels of GGT activities in hepatitis and normal sera

The Km and Vmax values for serum GGT enzyme of normal and hepatitis was estimated (table 2) by using Michael,s-Menten plots (figure1,table 2), the Km and Vmax values for GGT enzyme was increased in hepatitis patients compared with control groups.

Group	Km (mM)	Vmax IU/L
Normal	15.005	30.01
Hepatitis	28.49	56.99

Table(2):Km and Vmax value for GGT activity in both serum normal and hepatitis

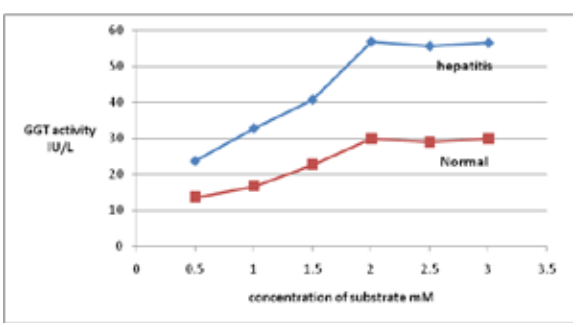
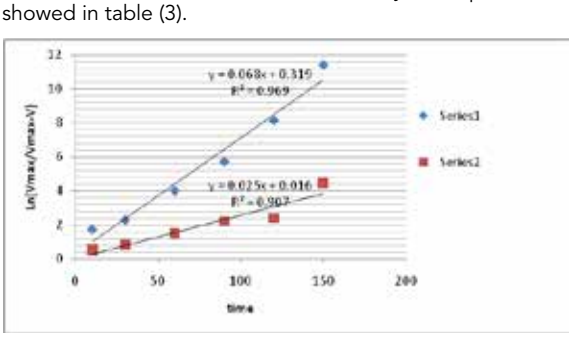


Figure (1):Michaeles-Mentin plot for both normal and hepatitis sera

Investigating the rate reaction kinetic mechanism and type of the order of the reaction, a pseudo first-order type were obtained in both normal and hepatitis figure (2) the K_1 and t1/2 were also obtained , discussing Hill coefficient (n) (figure 3) ,results shows a value of Hill coefficient(n) higher in hepatitis than in normal. Also, Arrehenius relationship(figure 4) revealed a lower level in the energy if activation in hepatitis reaction than that in normal,all thermodynamic parameters showed in table (3).



Figure(2): The pseudo first-order plot for GGT activity in serum normal and hepatitis

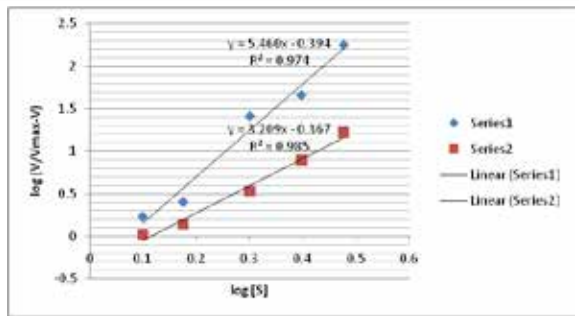


Figure (3): Hill coefficient (n) for GGT in serum normal and hepatitis

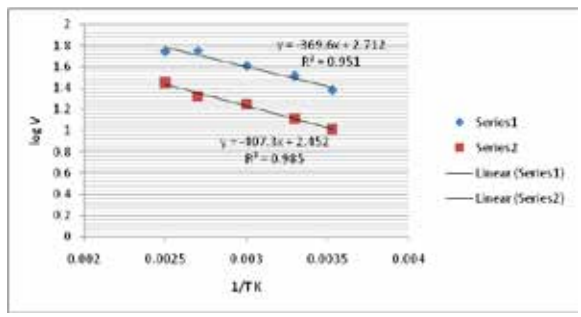


Figure (4): Arrhenius plot for GGT activity in serum normal and hepatitis

Group	$K_{1(min-1)}$	t1/2	(n)	Ea*	ΔH*	ΔG*	ΔS*
Normal	0.025	27.72	3.209	1854.84	1314.3	10642.27	-34.168
Hepatitis	0.068	10.661	5.460	1683.15	1142.62	8473.55	-26.851

Table (3) : All thermodynamic parameters of transition state for both normal and hepatitis.

Discussion:

The gamma-glutamyltransferases are the key enzymes in the so-called gamma-glutamyl cycle involving glutathione synthesis,the recovery of its constituents,and in the transport of amino acid(16),and this enzyme was shown to be a remarkable marker in the diagnosis. Investigating GGT enzyme,literatures concentrated on its activity determine and few research were published concerning rate reaction kinetics determination.

In liver disorder,studing gamma-glutamyltransferse activity revealed elevation in the enzyme activity(17,18). In our study,an elevation in GGT activity was also seen in hepatitis,one can conclude that as GGT in the liver was localized to the plasma membrane ,and these cells were damaged during their infection with hepatitis, therefore , this could be cause effusion of the cell fluids so that the GGT enzyme levels will secrets down the circulation.

Investigating the type of reaction order of GGT reaction fig(2),the data show that GGT reaction in both normal and hepatitis obey linearity and the straight line obtained indicates that the reaction is a first-order reaction . It was seen,that K_1 value is higher in hepatitis than that in normal,therefore,we can concluded that the disorder has its own effects in the equilibrium constant (Keq) of GGT reaction and then on ES-complex formation throughout increasing the association rate. The results,were also shows a decrese in t1/2 value in hepatitis than in normal upon which the authors can suggests to be due to acceleration in the enzyme activity because of the suggested damage occurs in hepatic cell(19). In this situation,a high secretion of GGT levels mean increasing the binding affinity of the enzyme to its substrate,this can be explained to be due to the increase in the isoforms of the enzyme,thus,the number of the binding sites will increase.

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The results presented in this work show significant change in (n) values in hepatitis comparing with that of normal group (table 3). These results suggested that allosteric conformational changes occurred in the GGT molecules upon binding to the substrate molecule in hepatitis patients (11).

Studying the energy of activation level for GGT enzymatic reaction (table3) avalue of Ea* was estimated from Arrhenius plot (fig 4),in which Ea* value found to be lower in hepatitis,this results indicate that the hepatitis may affect the mechanism of the GGT reaction (20). This predicts that there is another pathway for the enzyme-substrate complex (ES*) formation:

$E+S \leftrightarrow ES^* \rightarrow P+E$

The positive values of ΔH* for GGT in normal and hepatitis indicated that GGT reaction was endothermic and the heat content of the activated complex (ES*) were greater than that of the isolated species (E and S) (21). Also the results show that the ΔH* values for GGT reaction in hepatitis decrease,this indicated that the heat content of the activated complex;ES* in hepatitis were smaller than those in normal(22). The negative values of the ΔS* reflects a change to more ordered structure (23), and the positive charged values for the ΔG* for the enzyme-substrate reaction in both normal and hepatitis indicated that the active complex ES* formation required input of energy (24).

In conclusion,our experiments demonstrated that the thermodynamic studies of transition state changed in the mechanism of the ES-complex formation in the GGT enzyme reaction.

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