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Biochemical and Kinetic Study on Serum Adenosine Deaminase Enzyme in β-Thalassaemia

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Abstract

This study was conducted to investigate activity and some kinetic and thermodynamic parameters of adenosine deaminase (ADA)in serum in patient affected with β-thalassaemia and compared with that of healthy individuals .Serum AD levels were found to be significantly higher in patient with β-thalassaemia (98±9.15 IU/L) than in healthy individuals (22± 2.04 IU/L)the study was carried in optimum pH value 6.5 and 37c by which the enzyme possess highest activity .The study was concentrated to comprehensive determination of the Km,Vmax and rate reaction kinetics of the enzyme reaction in both normal and thalassaemic sera the pseudo first –order plot reflects both values of the first –order association constant (k_1) and the half life time ($t1/_2$) of the enzymatic reaction . The activation energy of the reaction (ES-Complex) formation was estimated using Arrhenius plot'

Introduction

In the thalassaemia the genetic disturbance leads to a reduction in the rate of synthesis of one of the normal polypeptide chains most commonly the β and α chain , and abnormal chains are not formed. In adult life an excess of β- chains which combine to form the unstable tetramer β_4 known as Hb-H. In β-thalassaemia, however there is no abnormal hemoglobin since although impaired β- chain synthesis leads to an excess of α – chains the latter are incapable of forming tetramers (1-3).Adenosin deaminase (ADA,EC3.5.4.4),a key enzyme in purine metabolism ,catalyzes the irreversible hydrolytic deamination of adenosine and 2-deoxyadenosine to inosine and 2"-deoxyinosine respectively (4). It is present virtually all human tissues, but the highest levels are found in the lymphoid system such as lymph nodes, spleen and thymus (5). Adenosine deaminase (ADA) is an essential enzyme for differentiation and proliferation of T

lymphocytes and the monocyte-macrophage systems and plays a significant role in the immune system (6) .Adeficiency of ADA activity is associated with a form of sever combined immunodeficiency diseases , while a 40-70 fold increase of its activity is associated with a form of chronic nonspherocytic hemolytic disease (7-9).Enzymes abnormalities have been reported also in some leukemia diseases, in acquired immunodeficiency syndrome ,in tuberculosis (10-12).

Materials and Methods

Chemicals

All chemicals used were of high analysis grad and purchased from BDH, FLUKA,otherwise it is specified .

Instruments

*Spectrophotometer , pectronic 601

*Philips pH-meter

*Centrifuge T-5

*Thermostatic water bath

Collection of samples

Blood analysis is usually done on venous of capillary blood .Seventy samples of blood were throughout the investigation of ADA enzyme activity . Fourty of these samples were of thalassaemia as diagnosed by physician , while the other are normal

Separation of serum

Five milliliters of blood has been collected and allowed standing at room temperature until it has clotted. The separated serum about 2-3mL, is centrifuge for removal of any suspended cells .

Assay of ADA activity

The protocol adopted from that of Giusti and Galanti (1968) in which the enzyme activity defined as "amount of enzyme that liberate one micromole of ammonia per one minute of the reaction " The activity was determined by using spectrophotpmetric analysis in which a colored complex is absorbed at 630nm, using series concentration of the substrate [S] fig(1)

Determination of Km and Vmax values of ADA enzymatic reaction

For the enzyme in the thalassaemic patients and normals the same protocol for the activity determination was used except by using different concentration of the enzyme substrate . Michaels –Menten

plots and Linweaver –Burk plot for enzyme was used , from which the Km and Vmax was determined fig(2,3)(13)

Determination of the rate association constant K1 and the t1/2

Using time cours the order of the enzymes, Rate of constant of the forward reaction (k1), and the half life times (t1/2) were determined

The same protocol of activity was used except by changing the incubation times fig(4,5).

Determination of the Activation Energy

The same protocol for ADA activity determination was used and the only difference is the use of various incubation temperature fig(6)

Results and Discussion

Determination of ADA activity in β- thalassaemic serum was determined quantitatively and compared with that of normal one using developed calorimetric method based on Giusti and Galanti (14). Data obtained revealed specific elevation of ADA activity in thalassaemic serum (98±9.15IU/L)in comparison with normal (22±IU/L)fig (1).Elevation of ADA activity can be explained to be due to affection of liver by thalassaemia and may resulted in a change of liver cells ,thalassaemic patient suffer from liver diseases and bone diseases ,as diagnosed by physicians , therefore, liver enzymes ADA and their structure can be affected in case liver cell damaged causing alteration in the enzyme activities. (15)Another choice of suggestion is that as the duration of diseases become so long this could lead to the damage of other specific tissues resulting in increasing level of ADA(16,17) .

Determination of Km and Vmax may produce a diagnostic parameter for thalassaemia syndrome type β- as the sera used in this research were of β- thalassaemic patient as diagnosed by physicians . Table(1) shows the determined values for Km and Vmax in normal and thalassaemic individuals, the importance of Km and Vmax is that their levelvariations can be presumed to be a diagnostic marker for abnormal cases . In the thalassaemic serum the Km values of the ADA is lower than normal individuals fig.(3) ,this will indicate that the affinity of the enzyme is greater in the case of the thalassaemia to its substrate, while Vmax values are higher in thalassaemic patient compared with normal individuals fig. (2) .This may be due to the changes in the ratio of isoenzumes in thalassaemic patient , or may be due to changes in the structure or conformation of the enzyme.

Investigating the rate reaction kinetic mechanism and type of the order of the reaction , pseudo first- order type fig .(4) were obtained in both normal and thalassaemia cases the value of K_1 and t1/2 table(2) were also obtained using the relation :

Ln(V max / V max-V) VsT T—time

From the above the value of K_1 is determined and value of t1/2 was calculated as :

t1/2 =0.693/K

The resulted showed, the forward rate constant (K_1) for ADA increased in thalassaemic serum table(2) fig.(4) ,so one can conclude that the diseases may affect the equilibrium constant (K_{eq}) of ES* complex formation throughout increasing the association rate constant (18,19)

$$\text{-----} \quad \ldots\ldots E+S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ES^*$$

$K_1[ES^*]=K_1[E][S]$

$K/ K_1\text{-}1=[E][S]/[ES^*]=K_{eq}$

K_1-= dissociation constant

These results also showed that the diseases affected (decreased) the half life time (t1/2)of ada enzyme reaction table (2) fig.(4), this may be due to the acceleration of activities by increasing the enzyme concentration (number of active sites) and the affinity of the enzyme to their substrate.

Studying the activation energy level for ADA enzymatic reaction table(3) was estimated from Arrhenius plot fig.(5) , in which Ea value found to be higher in thalassamia than in normal. This can be due to the highly affected mechanism of the reaction thalassamia. ADA must obtain a greater amount of energy barrier of the reaction to produce the product .This can be explained practically that the ADA enzyme concentration increase in the circulation due to the releasing of the enzyme from site sources (20-22).

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Table (1) : Km and Vmax values of Normal Thalassaemic SADA

Enzyme	thalasssaemia		Normal	
	Km(mM)	Vmax(IU/L)	Km(mM)	Vmax(1U/L)
ADA	0.5	95	0.9	25

Table (2) :Enzyme reaction parameters of Normal and Thalassaemic SAD

Enzyme	$K_i(min^{-1})$		t1/2	
	Normal	Thalassaemia	Normal	Thalassaemia
ADA	0.27	0.035	22	19.8

Table (3) :Activation energy of Normal and Thalassaemic SAD

Enzyme	Activation energy Ea	
	Normal	Thalassaemia
ADA	9617.8	17111

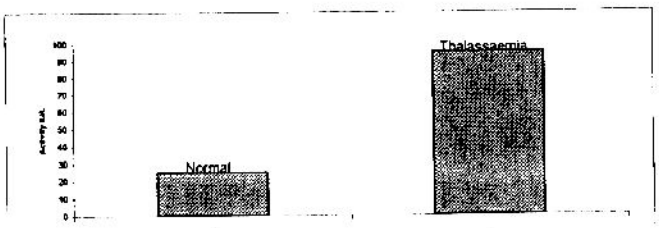

Fig.(1):Comparing ADA activity in thalassaemic and normal serum .

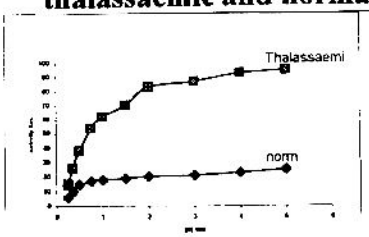

Fig.(1):Michaeles –Mentin plot for both normal and thalassaemic serum .

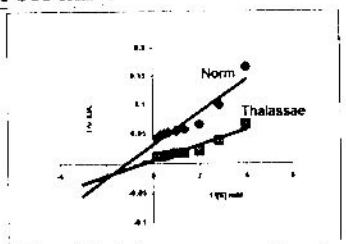

Fig.(3):Linweaver-Burk plot for both normal and thalassaemic serum .

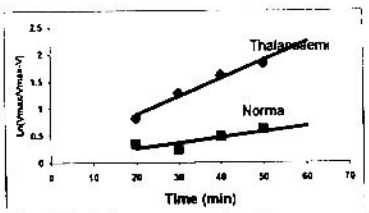

Fig.(4):The pseudo first order ploth for ADA enzyme for both normal and thalassaemic serum .

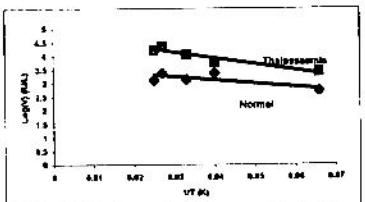

Fig.(5):Arrhenius plot for ADA enzyme for both normal and thalassaemic serum .

دراسة كيمو حيوية حرارية للأنزيم الأدينوسين دى امينس في مصل البيتا ثالاسيميا

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الخلاصة

يتحقق هذا البحث عن فعالية الادينوسين دى أمينيس وبعض الدوال الثرمودانميكية في المرضى المصابين ببيتا ثالاسيميا ومقارنتها بالأشخاص الأصحاء اذ وجد ان فعالية الادينوسين دى امينيس تزداد زيادة معنوية في مرضى البيتا ثالاسيميا (95±9.15IU/L) وهو اعلى من معدلاته في المصول الطبيعية والبالغة (25±2.6IU/L) . كما ابدى الادينوسين دى امينس قمة نشاطه في درجة أس الهيدروجيني المثلى 6.5 ودرجة حرارة 37c . ولقد ركزت الدراسة تعين قيم الحركية للانزيم مثل Km andVmax وعلى متابعة ميكانيكية التفاعل الأنزيمي ،اذ شملت الدراسة قيم معدلات ثابت التكوين (K1) مدة نصف العمر (t1/2) ، اذ تم استنتاج نمط ومرتبة التفاعل الأنزيمي واتضح انه يخضع للعلاقة الخطية مما يدل على كونه من المرتبة الأولى . وكذلك شملت الدراسة على تقدير مقدار طاقة التنشيط للمعقد الوسطي المتكون (ES) وباستخدام علاقة ارهينوس .