Kinetic Studies on ADA on Sera of Patients with Seronegative Arthritics, Hemolytic Anemia and Leukemia

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Abstract :

Kinetic studies was carried out on ADA activity in normal and patients with Seronegative Arthritics, Hemalytic Anemia and Leukemia . The optimum temperature was found 37C^{o} while the optimum pH was 6.5 in control and patients individuals , also it is found that the values of K_m were ($5x10^{-3}$, $6.2x10^{-3}$, $1.4x\ 10^{-2}$, and $12.4x\ 10^{-2}$M) and of V_{max} were (36.23, 37.3, 52.8 and 56.5 mM .min^{-1}.mg^{-1}) respectively.

Key Words : ADA , Kinetic studies , Michaelis-Menten constant Km

Introduction :

Adenosine deaminase (ADA) is considered to be one of the enzymes involved in the catabolism of purine base of the purine salvage pathway [1-2] , adenine part of adenosine can be converted to uric acid [3] . ADA is widely distributed in microorganisms , plant and animal in mammalian tissues and fluid including those of human [4-7] .

A characteristic distribution of ADA activity in human sera of normal individuals was observed at 37^{o}C was 10-25 IU/L[8] . Elevated of enzyme activity may observed in many diseases [9-13] . Recent study achieved by AL-Assi W. [14] . Showed that ADA activity was 36 ±4 IU/L , 56.0 ±3.3 IU/L , 68.4 ±2.1 IU/L in patients with Seranegative Arthritis , Hemayltic Anemia and Leukemia , respectively .

The rate of reaction catalyzed by an enzyme is directly proportional to the enzyme concentration . Measuring reaction rate under standard conditions of substrate ,pH and temperature serve as a measure of enzyme activity and concentration[15] . K_m is the Michaelis-Menten constant which expresses the substrate concentration at which the reaction rate has half of its maximum value . If the concentration of a substrate is increased while all other conditions are kept constant , the measured initial velocity Vi , increases to a maximum value V_{max} [16] . Therefore the present study included several experiments in order to determine optimum conditions measurement of ADA activity in patients with Seronegative Arthritis , Hemolytic Anemia and Leukemia and compared with those of normal individuals .

Samples

Serum was collected from 48 patients admitted to Tikrit General Hospital , based on clinical manifestation and laboratory results , samples were divided into:

Group I : the Seronegative Arthritics (12 female , 8 male)

Group II : the Hemolytic Anemia (7 female , 8 male)

Group III : the Leukemia (7 femal , 6 male) .

A 4th group consisted to (12 female , 18 male) normal individuals were used as control . The whole blood samples were left for 20 min at room temperature . After coagulation the sera were separated by centrifugation at 3000Xg for 10 min then aspirated carefully [16] .

Methods

1-The activity of ADA was measured according to Galanti and Giustic method[3] :

The absorbance was measured at 630 nm and the enzyme activity was expressed as International Unit / Liter .

Table (1) ADA activity measurement procedure

No.	Pipette successively in test tubes	Sample (ml)	Sample Blank (ml)	Standard (ml)	Reagent (ml)
1-	Phosphate buffer 50mmol/L , pH 6.5	-	-	-	1.00
2-	Buffered adenosine solution 4µ mol /L	1.00	1.00	-	
3-	Ammonium sulphate standard solution 75µmol/L	-	-	1.00	-
4-	Serum	0.05	-	-	-
5-	Distilled water	-	-	0.05	0.05
Mixed and cap tubes , incubated for 60min in a 37C water- bath					
6-	Phenol nitroprusside solution : phenol (106µmol/L), nitroprusside (0.17µmol/L)	3.00	3.00	3.00	3.00
7-	Serum	-	0.05	-	-
8-	Sodium hypochlorite solution : hypochlorite (11µmol/L) , NaOH (125µmol/L)	3.00	3.00	3.00	3.00
Mixed , incubated for 30min in a 37C water bath , measure absorbance against water bath at 630nm					

$$\text{Activity of ADA IU/L} = \frac{\text{A sample - A sample Blank}}{\text{A standerd - A reagent Blank}} \times 0.15 \times \frac{1}{60} \times \frac{1000}{0.05}$$

$$= \frac{\Delta \text{ A sample}}{\Delta \text{ A standerd}} \times 50$$

$$= \text{IU/L}$$

2- Effect of substrate concentration on ADA activity :
Michaclis –Menten constant (K_m) and maximum velocity (V_{max}) of ADA were determined by using the same procedure in (1) above . Hence the adenosine concentrations as substrate were prepared as follows (0.0012, 0.005 , 0.01 , 0.015 ,0.02, 0.025 mM) . ADA activity at each concentration was measured and plotted versus adenosine concentration .
The K_m and V_{max} of ADA reaction with adenosine were calculated according to lineweaver-Burk method [16] using the following relationship :

$$\frac{1}{V} = \frac{1}{Vmax} + \frac{Km}{Vmax} \times \frac{1}{[s]}$$

3- Effect of Incubation temperature on ADA activity :
The activity of ADA was measured at different incubation temperatures (0,5,10,15,20,25,30,35,37,40,50,60C^o) by using optimum pH and substrate concentration . The activity was plotted versus temperature .

4- Effect of pH on ADA activity :
By using the optimum substrate concentration , the activity was measured at different pH (4,5,6,6.5,7,8,9) .ADA activity was plotted versus pH .

Results and Discussion :

Several studies was showed high serum ADA activity in patients with many diseases and could be a good marker for them [3,9,12-14,17-21]. The present study was planned to clarify ADA kinetics in Seronegative Arthritics, Hemolytic Anemia and Leukemia patients in comparison to normal individuals , Km is the michaelis-Menten constant , it expresses the substrate concentration at which the reaction rate has half of its maximum value[16] . Kinetic constant of ADA catalyzed the conversion of adenosine to inosine were found to be readily obtained by analyzes curve of a single reaction by conversional initial velocity analysis[22] . In a study carried out by Al-Dahhan .J.[21], K_m and V_{max} were actually found to be $11x10^{-3}$ mM and 17.54 $mM.min^{-1}$ respectively in the serum of normal indevduals , whereas a study by spectar T [22] showed that the values were 29.3µM and 1.27µ $mol.min^{-1}$ respectively .Al-Obadi A.[12] stated that K_m and V_{max} were 3.45 $x10^{-3}$ M and 25mM/min respectively in Cerebrospinal fluid of meningitis in children , while Al-Assi W. [13] found that they were ($8x10^{-3}$M and 4.4m $M.min^{-1}.mg^{-1}$), ($10x10^{-2}$M and $3mM.min^{-1}.mg^{-1}$),($13x10^{-3}$M and $2.25mM.min^{-1}.\ mg^{-1}$), ($15x10^{-3}$M and $19mM.min^{-1}.\ mg^{-1}$) in normal, Anima, Rheumatoid Arthritis and in both respectively.
In our study we found that the values of K_m and V_{max} were ($5x10^{-3}$ M and 36.23 $mM.min^{-1}.mg^{-1}$) , ($6.2x10^{-3}$ M and 37.3 $mM.min^{-1}.mg^{-1}$), ($1.4x10^{-2}$ M and 52.8 $mM.min^{-1}.mg^{-1}$), (12.4 $x10^{-2}$ M and 56.5 $mM.min^{-1}.\ mg^{-1}$) in normal, Seronegative Arthritis, Hemolytic Anemia and Leukemia respectively theses results are indicated in Fig (1) , (2) , (3) and (4) repectively .

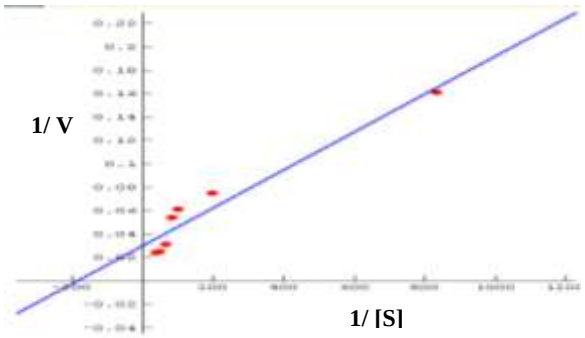

Fig (1) : Effect of adenosine concentration on ADA activity in normal as in lineweacer-Burk plot

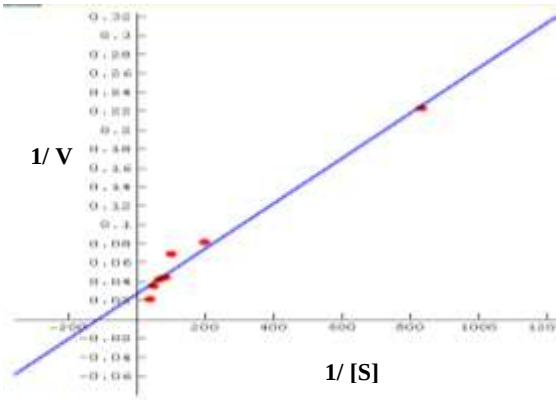

Fig (٢) : Effect of adenosine concentration on ADA activity in Seronegative Arthritics as in lineweacer-Burk plot

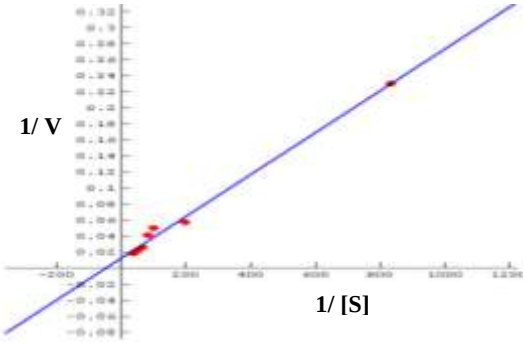

Fig (3) : Effect of adenosine concentration on ADA activity in Hemolytic Anemia as in lineweacer-Burk plot

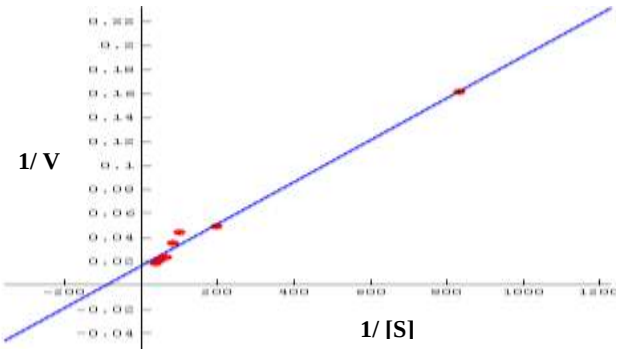

Fig (4) : Effect of adenosine concentration on ADA activity in Leukemia as in lineweacer-Burk plot

The effect of substrate concentration (adenosine) on the velocity of the reaction of ADA from normal and patients with Seronegative Arthritics , Hemolytic Anemia and Leukemia indicate that optimum adenosine concentration was (0.025 mM) [14]. The effect of temperature on the activity of an enzyme can easily shown by measuring the rate of enzyme – catalyzed reaction at several temperatures , over a limited range of temperature, the velocity of the reaction catalyzed by an enzyme increases as temperature rises. Fathomer, there is an optimal temperature at which the reaction is in high velocity, generally, the catalytic activity result from active tertiary or quaternary structure of the enzyme that binds to the substrate thus , ADA activity in normal, Seronegative Arthritis, Hemolytic Anemia and Leukemia at 37C^{o} is higher that its activity using other temperatures, in addition , above this degree, ADA activity decreases due to disruption of the tertiary structure and thermal denaturation of the proteinaceous enzyme structure, while below 37^{o}C. ADA activity decreases due to insuffient energy that required to perform enzyme-substrate complex [23] these results are shown in Fig(5) ,(6) , (7) and (8) respectively .

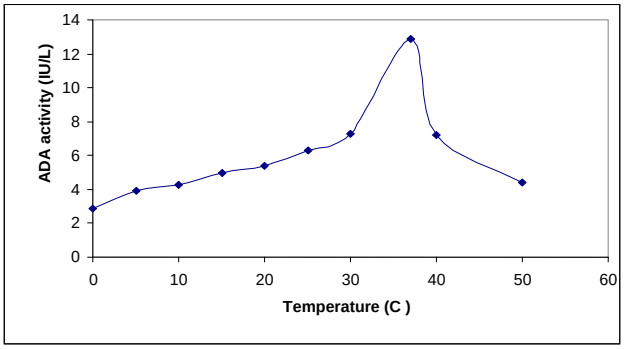

Fig (٥) : Effect of incubation temperature on ADA activity in normal individuals

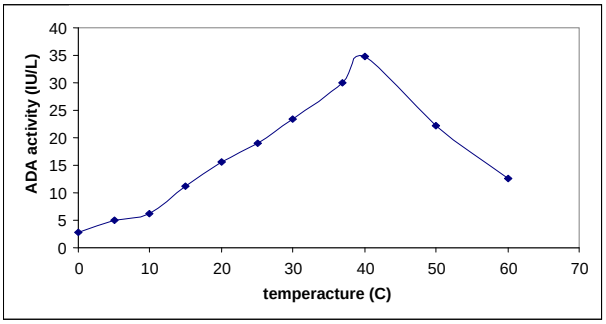

Fig (٦) : Effect of incubation temperature on ADA activity in seronegative Arthritics

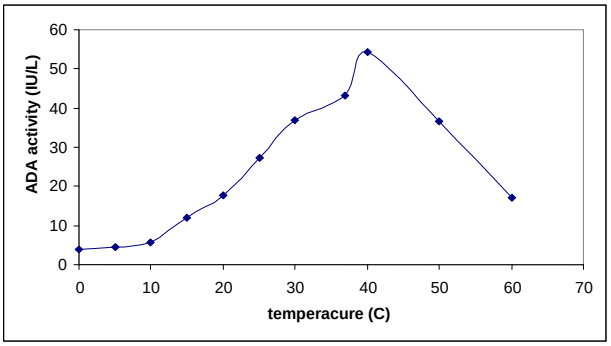

Fig (٧) : Effect of incubation temperature on ADA activity in Hemolytic Anemia

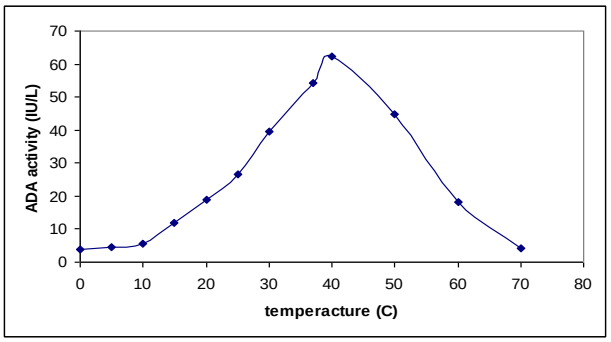

Fig (٨) : Effect of incubation temperature on ADA activity in activity in Leukemia

The effect of pH on the activity of ADA in normal Seronegative Arthritics , Hemolytic Anemia and Leukemia can be illustrated in Fig (9), (10), (11) and (12) respectively , which indicates that the optimum pH for ADA is 6.5.Generally at pH below 6 , enzyme protonates and loses its negative charge thus loss its activity to bind to the substrate , whereas , at pH higher than 6 , the substrate ionizes and loses its positive charge , thus loose its affinity to bind to the enzyme to form enzyme – substrate complex , and decrease the reaction velocity [24].

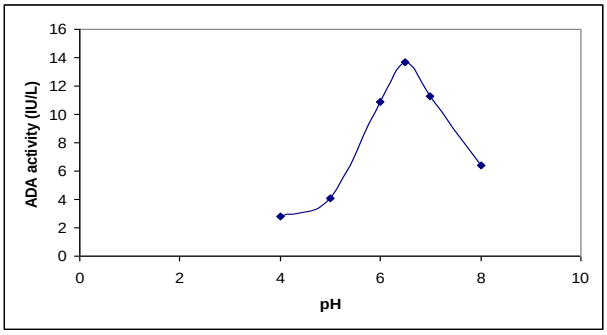

Fig (٩) : Effect of pH on ADA activity in activity in normal individuals

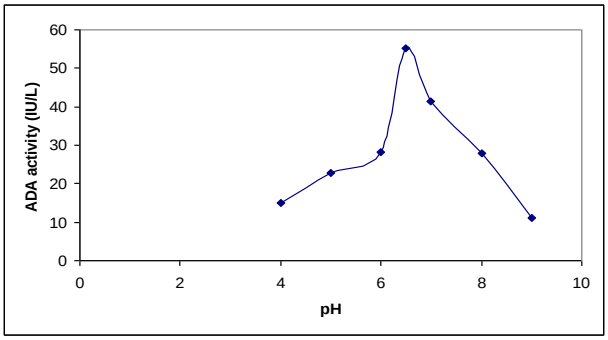

Fig (11) : Effect of pH on ADA activity in activity in Hemolytic Anemia

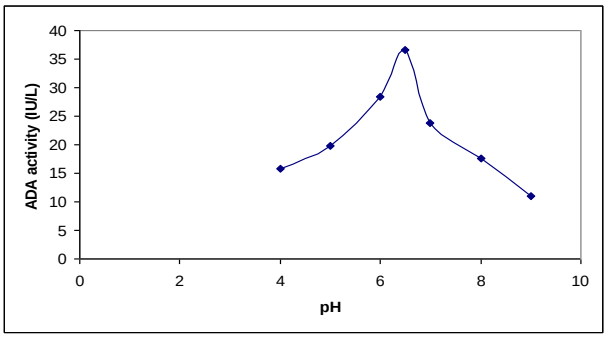

Fig (10) : Effect of pH on ADA activity in activity in Seronegative Arthritics

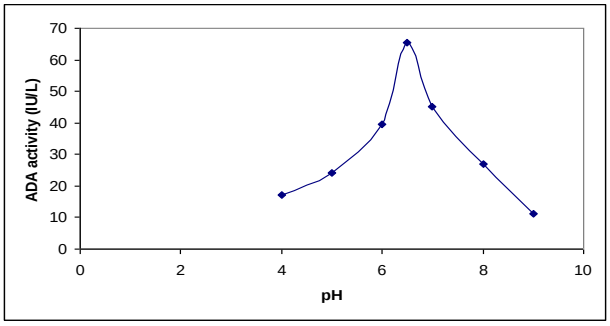

Fig (12) : Effect of pH on ADA activity in activity in Leukemia

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دراسة مركبات أنزيم الأدينوسين دي أمنيز في أمصال المرضى المصابين بالتهاب المفاصل الرثوي سالب المصل وفقر الدم التحللي وابيضاض الدم

سوزان جميل علي

قسم الكيمياء ، كلية التربية ، جامعة تكريت ، تكريت ، العراق

الخلاصة

تضمن البحث دراسة حركية انزيم الادينوسين دي امنيز ADA في المصل الطبيعي ومصل المصابين بالتهاب المفاصل الرثوي سالب المصل Rh^-، فقر الدم التحللي واللوكيميا. حيث وجد أن أقصى فعالية للأنزيم عند درجة حرارة ٣٧ مْ واس هيدروجيني ٦,٥ في كل الحالات، كذلك درست قيم الثوابت الحركية للانزيم في المصل ووجد ان قيم ثابت مكيلس- منتن Km كانت (٥×١٠$^{-٣}$، ٦,٢ ×١٠$^{-٣}$، ١,٤×١٠$^{-٢}$، ١٢,٤ ×١٠$^{-٢}$ M) على الترتيب، إما قيم السرعة القصوى V_{max} فكانت ($mM.min^{-1}.mg^{-1}$ 36.23، $mM.min^{-1}.mg^{-1}$ 37.3، $mM.min^{-1}.mg^{-1}$ 52.8, $mM.min^{-1}.mg^{-1}$ 56.5) على الترتيب .