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Kinetic and Thermodynamic Study of the Activity of AST Analogues Purified From the Blood of Patients with Diabetes Mellitus

Jassim Mohammed Dahir
Tikrit University - College of Education for Pure Sciences - Chemistry Department

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Annotation: 1- The aminotransferase enzyme (AST) activity was measured in the blood of diabetic patients and healthy people. The results showed increased activity of (AST) in patients' blood compared to healthy people.

2- (AST) was purified from the blood of diabetic patients using gel filtration chromatography and Sephadex G-25 filtration gel

3- Two isomers of (AST) were separated from the blood of diabetic patients using ion exchange chromatography and using DEAE-Sephadex X A-50 resin. 4- The kinetic properties of (AST) isomers were studied, and it was found that the isomers are subject to the Michaelis-Menten equation, as the optimal concentration of the basic material (aspartic acid) for each of the isomers was (mole.dm-3 10-3 x 166.5).

5- It was observed that the two analogues obey the Arrhenius equation up to (37) ˚C, and Ea and Q10 were assigned to each of the analogues.

6- The thermodynamic functions of the activity of the amino group transfer enzyme (AST) were calculated through the Van't Hoff and Arrhenius equations for the standard and transition states.

Keywords: Aspartate aminotransferase, Kinetic Properties of AST, Thermodynamics of AST.

1. Introduction

Aspartate aminotransferase is a widespread enzyme in various body tissues and indicates liver function tests. It is also found in the heart, pancreas, and kidneys. Its level in the blood varies from person to person [1]. This enzyme's activity level in the blood of healthy people is low compared to those affected. It was found that the level of activity of this enzyme in healthy people ranges between (40-5) international (units/litre), and its highest activity is in the liver[2]. When the liver is damaged or diseased, AST's activity level increases above the expected level for a period of(10-6) hours after the injury[3, 4]. It was found through studies conducted on humans that the activity of the AST enzyme in the blood serum of a person with diabetes increases until it reaches (138) international units/litre [5]. Thus, measuring AST activity is a laboratory measure in clinical medicine to assess the degree of infection with liver diseases and some other diseases [6-8]. The first method used to measure enzyme activity was the chromatographic separation method of the product, which is glutamate, formed from the reaction of (alpha-ketoglutarate with aspartate in the presence of AST) [9,10]. **Isoenzymes of Aspartate aminotransferase** Isoenzymes are generally defined as enzymes that have the same function but differ in physical, chemical and kinetic aspects [11]. It was found that the AST enzyme has isoenzymes ranging in number from (1-8) with the same function and different in their molecular weights [12]. Three isoenzymes of AST were separated from the blood serum of patients with several liver diseases and from the blood of healthy people using DEAE - Sephadex A - 50 [12]. Three isoenzymes of AST were also separated from the blood serum of normal humans using a simple and sensitive chromatography method based on the use of negative ion exchange gel type DEAE - Sephadex A - 50, and the kinetics of these isoenzymes were studied [13-16]. **Kinetic Properties of Aspartic amino transferase** These enzymes are catalysts for reactions and are characterized by the general properties of catalysts. The speed of the enzymatic reaction is affected by a set of factors, the most important of which are temperature, pH, concentration of the substrate, enzyme concentration, concentration of the resulting substance, and the presence of inhibitors. To study the effect of any of them, the enzymatic reaction is performed several times to change a certain factor, and the reaction speed is measured each time while keeping the rest of the other factors constant without change. Each enzyme has an optimum temperature at which the reaction speed is as high as possible; then, the speed decreases with increasing temperature. The optimum temperature for any enzyme depends on the period of time to which the enzyme was exposed [17]. Blood serum was found to not change the enzyme activity when frozen or cooled using dry ice and when stored in the refrigerator for two weeks at a temperature of (0-5 ˚C). While the enzyme is destroyed at a temperature of (100 ˚C) [18,19]. The effect of temperature in increasing the reaction rate is likely to increase the internal energy of the molecules based on the Arrhenius equation, which determined the dependence of enzymatic reactions on the temperature coefficient (Q_{10}). The temperature coefficient (Q_{10}) was defined as the factor that increases the reaction rate when the temperature rises every ten degrees Celsius. The enzyme reaction rate is also affected at a specific range of pH values, as we find that no reaction occurs at a specific range of pH values, while we see different reaction rates at other pH values. For example, the enzyme purified from human serum works at an acidic function ranging between (8 - 7.4) [20]. The other influential factor is the enzyme concentration [20]. **Thermodynamics of AST** Thermodynamics deals with studying the properties of groups in equilibrium conditions and is not concerned with the effects of the time factor. Through it, we obtain accurate relationships between different measurements and provide the basis for predicting the effects of temperature, pressure, and concentration on chemical equilibria. It does not depend on the molecular structure or the reaction mechanism by which groups reach equilibrium. It deals with the study of the heat absorbed or lost when a chemical reaction occurs a phase change, or when a solution is diluted. Exothermic reactions generate heat and have negative values for ΔH and ΔG . Endothermic reactions absorb heat and have positive values for $H\ \Delta$ and $G\ \Delta$. There is a third factor, $S\ \Delta$, the entropy change that completes the thermodynamic quantities and is considered a function of the state and a measure of the regularity or irregularity of the group. In a chemical reaction, the base material needs much energy to reach

the transition state, and the energy required to form the product is reduced in the presence of the enzyme. As with the rest of the cofactors, the AST enzyme does not change the position of the chemical equilibrium of the reaction. The reaction proceeds in the same direction in the presence of AST but faster. However, spontaneous, uncatalyzed reactions can occur in the absence of AST, leading to different results [21,22].

2. Materials and Methods

The materials used to measure the activity of the aminotransferase enzyme (AST) and purify the enzyme analogues were obtained from different sources. From the English company (BDH), we received (L-Aspartic acid, potassium dihydrogen phosphate, and potassium mono hydrogen phosphate); from the Fluka company, we obtained Keto glutarate - α; and from the Pharmacia fine chemicals company, we obtained DEAE - Sephadex A-50.

Specimens (10) blood serum samples were collected from people with diabetes, and (10) blood serum samples were collected from healthy people after ensuring they did not have diabetes. 2 cm^3 of serum was collected in a clean, dry tube. This sample is used only once, i.e. it is not stored for the next day, as the enzyme activity is measured immediately after taking it.

Solutions used: [23]

1. Phosphate buffer solution (0.1 mol / L)

Prepared by dissolving (2.7 g) (Potassium dihydrogen phosphate) KH_2PO_4 with (11.3 g) of (Potassium monohydrogen phosphate) K_2HPO_4 in a litre of distilled water, thus obtaining a buffer solution with a pH of (7.4), then stored in the refrigerator at a temperature of (4)˚C.

2. Basic materials solution:

It includes aspartic acid (166.5 mmole.dm^{-3}) and alpha-ketoglutarate acid (1.66 mmole.dm-3). It was prepared by dissolving (2.216 g) of aspartic acid in (20 ml) of sodium hydroxide at a concentration of (1 mole.dm^{-3}). The solution was adjusted to (pH 7.4) using drops of sodium hydroxide (1 mole.dm-3), then (0.024 g) of alpha-ketoglutarate was added to the solution, and the pH was adjusted again to (7.4) using drops of sodium hydroxide. The volume was completed to (100 ml) with phosphate buffer solution, and the solution was stored at (15)˚C in (5 ml) bottles.

3. Standard stock pyruvate solution (20 mmole.dm^{-3})

Prepared by dissolving (0.22 g) of sodium pyruvate in (100 ml) of buffer solution and stored at (15)˚C in (100 ml) bottles.

4. Standard pyruvate solution used (mmole.dm-3 4) DNPH

Prepared by diluting (ml 1) ml of the solution prepared in step (3) in (ml 5) of phosphate buffer solution and stored at (15)˚C. Prepared weekly.

5. 2,4-Dinitrophenylhydrazine solution (2mmole.dm^{-3})

Prepared by dissolving (0.0198g) of (2,4-Dinitrophenylhydrazine) in (ml 10) of concentrated hydrochloric acid, complete the volume to (ml 100) with distilled water and stored in an opaque bottle at room temperature.

6. Sodium hydroxide solution (0.4mole.dm^{-3})

Prepared by dissolving (g 16) of sodium hydroxide in a litre of distilled water.

Method of work:

The activity of the amino group transfer enzyme (AST) is measured according to the following:

Preparation of solutions

1. Preparation of the test solution

It is prepared by adding (0.5 ml) of the basic materials solution (aspartate acid with alpha-ketoglutaric acid) and incubating the tube in a water bath at (37) 5°C for three minutes, then adding (0.1 ml) of serum and mixing the contents of the tube and then placing it in the water bath for (60) minutes. The reaction is stopped after the incubation period ends by adding (0.5 ml) of (DNPH).

2. Preparation of the control solution

It is prepared by mixing (0.5 ml) of the basic materials solution with (0.5 ml) of DNPH solution, then adding (0.1 ml) of serum.

3. Preparing the standard solution

It is prepared by mixing (0.1 ml) of the standard pyruvate solution with (0.4 ml) of the basic materials solution, then adding (0.1 ml) of distilled water, then adding (0.5 ml) of the DNPH solution.

4. Preparing the equivalent solution

It is prepared by mixing (0.5 ml) of the basic materials solution with (0.1 ml) of distilled water, then adding (0.5 ml) of the DNPH solution. The mixture is mixed well.

5. After completing the preparation of the tubes mentioned above, leave them at room temperature for (20) minutes, then add (5 ml) of sodium hydroxide with a concentration of ($0.4 mol.dm^{-3}$) to each tube and mix well, then leave them for another ten minutes, then measure the colour absorbance at the wavelength (510 nm).

Separation and purification of (AST) analogues from the serum of healthy people and patients with diabetes

The harmful ion exchange chromatography column method DEAE - Sephadex A-50 was used to separate the amino group transfer enzyme AST analogues from the serum of healthy people and patients with diabetes.

Method of work:

1. A glass column with a diameter of (1.5 cm) and a length of (20 cm) was used. A little glass wool was placed at its end to prevent the gel particles from leaking out of the column. The suspended gel solution was poured into the column slowly and homogeneously to avoid the formation of Air bubbles until the gel height reached (8 cm). Then, the gel column was washed with quantities of phosphate buffer solution and acid exchange (7.4).

2. (1 ml) of serum is added slowly over the surface of the gel, and the serum is left in the column for (5-3) minutes to absorb into the gel column.

3. The separation process begins by using (18 ml) of phosphate buffer solution and collecting (10) tubes of the leached parts with a volume of (2 ml). Separating using phosphate buffer solution containing graded concentrations of sodium chloride and the process is done as follows: By adding (12 ml) of phosphate buffer solution containing graded concentrations (0.4, 0.3, 0.2, 0.1) mol/L for each step (and prepared by dissolving a specific weight of sodium chloride in (50 ml) of phosphate buffer solution with a pH of (7.4) to a column for the first three concentrations except for the last concentration (0.4 mol/L) by adding (24 ml) and collecting (12) tubes of the leached parts.

Calculating the activity of the aminotransferase enzyme (AST): The activity of the enzyme is measured by the amount of pyruvate formed per minute for every (1000 ml) of serum according to the following equation:

$$\text{Enzyme activity (AST)} = \frac{Reading\ the\ Test - Reading\ the\ Control}{Reading\ the\ analogy - Reading\ the\ equivalent} \times 0.4 \times \frac{1}{6} \times \frac{1000}{0.1}$$

Or $$\text{Enzyme activity (AST)} = \frac{Reading\ the\ Test - Reading\ the\ Control}{Reading\ the\ analogy - Reading\ the\ equivalent} \times 67$$

Where:

0.4: The amount of pyruvate formed.

60: The time per minute.

1000: The serum.

The enzyme activity is expressed in the international unit, which is defined as the amount of enzyme that releases ($1X10^{-6}$ $mol.dm^{-3}$) of the materials resulting from the reaction per minute and under optimal experimental conditions.

The studies kinetic

The kinetic properties of the amino group transfer enzyme isomers (II, I) were studied in the mature parts as follows.

1. Effect of the substrate (aspartic acid)

The effect of different concentrations of the substrate (aspartic acid) on the reaction rate of the enzyme isomers (II, I) was studied using various concentrations of aspartic acid (250, 200, 166.5, 140, 120, 100, 80, 40, 20) $mmole.dm^{-3}$ to calculate the optimum concentration of the enzyme isomers (II, I). The optimum concentration is calculated by drawing the relationship between the reaction rate and the substrate concentration. At this point, the reaction rate reaches its maximum speed.

2. Effect of pH

The effect of pH on the reaction rate of the isomers (II, I) was studied. Different values of the acid function of the phosphate buffer solution (8.0, 7.6, 7.4, 7.0, 6.8) were used in the presence of the base solution with a concentration of mmole.dm-3 (166.5). The reaction was carried out in a water bath at a temperature of (37) ˚C, and the tubes were incubated for (60) minutes.

3. Effect of temperature

This experiment was conducted to study the effect of temperature on the reaction rate of amino group transfer enzyme (AST) isomers (II, I) and to measure the maximum speed where the reaction was carried out at different temperatures (60, 50, 37, 10, 5)^{0}C, in the presence of phosphate buffer solution with acidity function (7.4), different concentrations (166.5, 80, 40 $mmole.dm^{-3}$) of the base material solution. The activity of enzyme isomers (II, I) was measured, and the relationship between the reaction rate of each enzyme isomer (II, I) and temperature was drawn.

Kinetic studies of the reaction order of the analogues (II, I)

The reaction order of the analogues of the amino group transfer enzyme (AST) (II, I) was determined by following the reaction using a phosphate buffer solution with a pH of (7.4) with a solution of the substrate (aspartic acid) at different concentrations (166.5, 80, 40) $mmole.dm^{-3}$. The reaction mixture was incubated for various periods (60, 45, 30, 15) minutes and at a temperature of (37) ˚C. The relevant equations were applied.

Thermodynamic studies of the analogues (II, I)

Thermodynamic coefficients for the standard and transition states were calculated using the Vant Hoff and Arrhenius equations, respectively.

3. Results and discussion

Measuring the activity of the aminotransferase enzyme (AST) in the serum of healthy and diabetic patients

(20) samples were tested, (10) of which were diabetic, and the enzyme activity level reached (26-19) international units/litre. And (10) cases of healthy individuals and the enzyme activity level in healthy individuals reached (19-1) international units/litre. It was noted when comparing the level

of (AST) activity in the serum of diabetic patients with its level in the serum of healthy individuals that an increase occurred in the level of patients more than in healthy individuals (39).

1. Kinetic studies of (AST) isomers (II, I)

1.1. Optimal concentration of the basic substance (aspartic acid)

Table (1) represents the activity values of the AST enzyme at different concentrations of aspartic acid for the isomer (I) purified from the serum of diabetic patients.

[Aspartic acid] (mmol.dm^{-3})	Activity AST (IU / L)
20	5
40	9.3
80	14.2
100	20
166.5	25
200	18.1
250	4

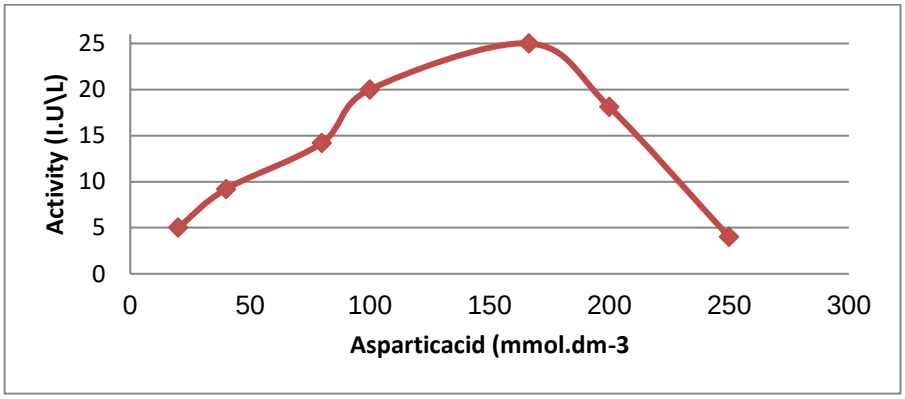


Figure (1) represents the relationship between different concentrations of (aspartic acid) and the reaction rate of the isomer (I) purified from the serum of patients with diabetes.

Table (2) shows the activity values of the AST enzyme at different concentrations of aspartic acid of the (II) analogue purified from patients with diabetes.

[Aspartic acid] (mmol.dm^{-3})	Activity AST (IU / L)
20	5.0
40	9.2
80	15.1
100	18
166.5	24.0
200	17.0
250	3.0

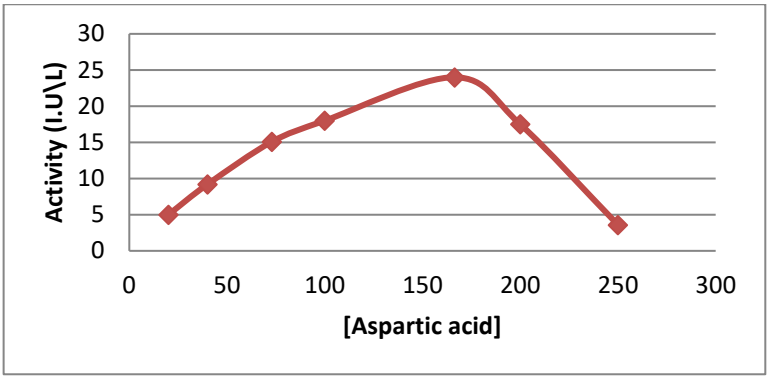


Figure (2) represents the relationship between different concentrations of (aspartic acid) mmol: dm-3 and the isomer (II) reaction rate purified from the serum of patients with diabetes.

Table (3) shows the activity values of the AST enzyme at different concentrations of aspartic acid for the isoform (I) purified from the serum of healthy subjects.

[Aspartic acid] (mmol.dm^{-3})	Activity AST (IU / L)
20	4.0
40	8.3
80	12.1
100	15.2
166.5	18.5
200	16.0
250	1.3

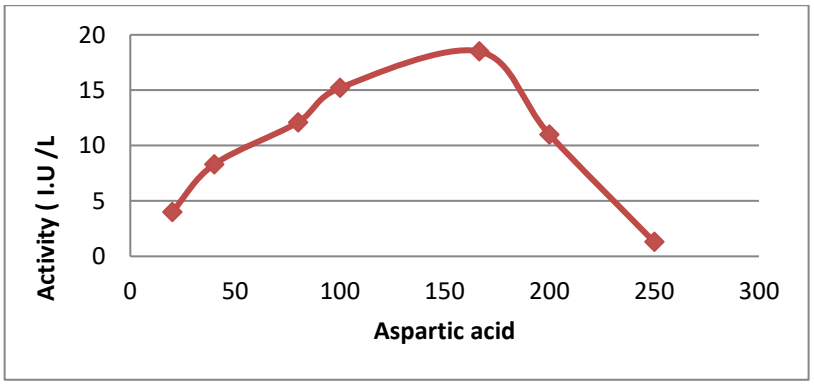


Figure (3) represents the relationship between different concentrations of (aspartic acid) mmol: dm^{-3} and the reaction rate of the isomer (I) purified from the serum of healthy subjects.

Table (4) shows the activity values of the AST enzyme at different concentrations of aspartic acid for the analogue (II) purified from the serum of healthy subjects.

[Aspartic acid] (mmol.dm^{-3})	Activity AST (IU / L)
20	3
40	5.3
80	7.5
100	8
166.5	13.2
200	4.3
250	1.0

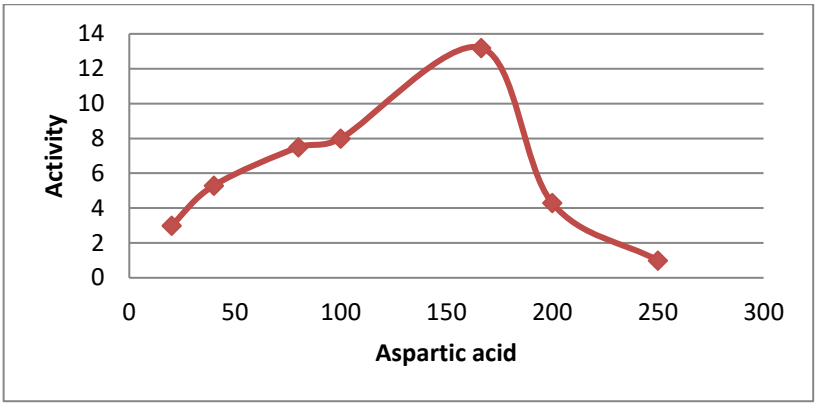


Figure (4) represents the relationship between different concentrations of (aspartic acid) mmol dm^{-3} and the reaction rate of the isomer (II) purified from the serum of healthy subjects.

1.2. The effect of the acidic pH function on the reaction rate of the aminotransferase (AST) isomers (I, II).

Tables (5) and (6) show the change in enzyme activity values versus the change in pH while keeping other factors constant.

Table (5) shows the AST enzyme activity values at different pH for the isomer (I) purified from the serum of patients with diabetes at a concentration of 166.5 mmol.dm^{-3}.

pH	Activity AST (IU / L)
6.8	5.5
7.0	8.2
7.4	14
7.6	8.5
7.8	2.0
8	1.0

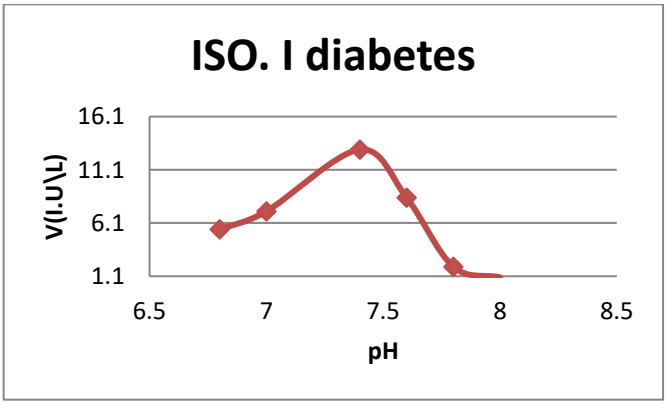


Figure (5) Effect of pH on the reaction rate of the isomer (I) purified from the serum of patients with diabetes.

Table (6) shows the activity values of the AST enzyme at different pH for the isomer (II) purified from the serum of patients with diabetes at a concentration of 166.5 mmol.dm^{-3}.

pH	Activity AST (IU / L)
6.8	7
7.0	12.2
7.4	17.5

7.6	10
7.8	5.3

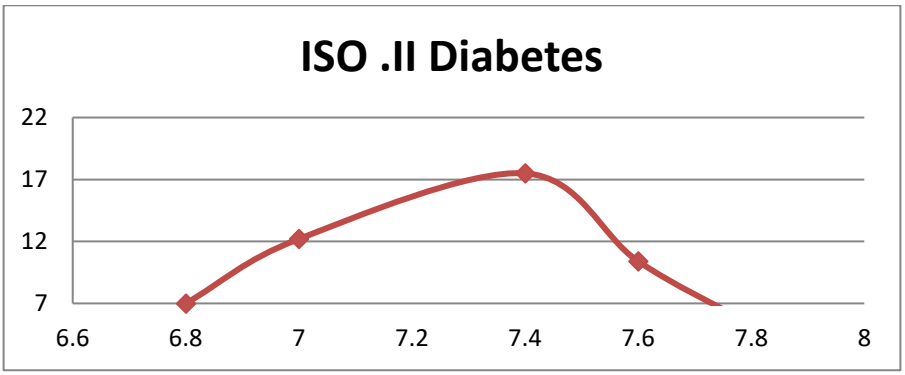


Figure (6) represents the effect of changing the acid function on the reaction rate of the isomer (II) purified from the serum of patients with diabetes.

1.3. Effect of temperature on the reaction rate of amino group transfer enzyme (AST) analogues (I, II) and the maximum speed Vmax

a. General relationship

Temperature affects the ionisation of groups on the surface of the enzyme and the substrate. Since the enzyme is a complex protein molecule, its catalytic activity is affected by high temperatures, which leads to the loss of the enzyme's structural composition. The enzyme cannot perform its active role when the temperature rises. The study showed that increasing the temperature after (37) ˚C leads to a disruption in the reaction rate until it reaches a degree less than optimal at high temperatures [24].

Table (7) shows the values of the activity of the AST enzyme at different temperatures at a concentration of (40 mmol.dm^{-3}) for the analogue (I) purified from the serum of patients with diabetes.

Temp.(C^0)	Activity AST (IU / L)
5	1.2
10	3.0
20	4.2
37	8.0
50	5.5
60	4.0

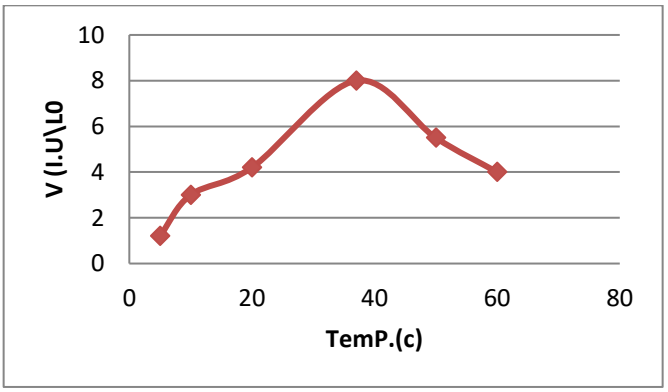


Figure (7) represents the effect of temperature on the reaction rate of the isomer (AST) (I) at a concentration of (40 mmol.dm^{-3}) and the optimum temperature for the rate.

Table (8) shows the activity values of the AST enzyme at different temperatures at a concentration of (80 mmol.dm^{-3}) for the isomer (I) purified from the serum of patients with diabetes.

Temp.(C^0)	Activity AST (IU / L)
5	4
10	6.3
20	7.5
37	10
50	7.1
60	5

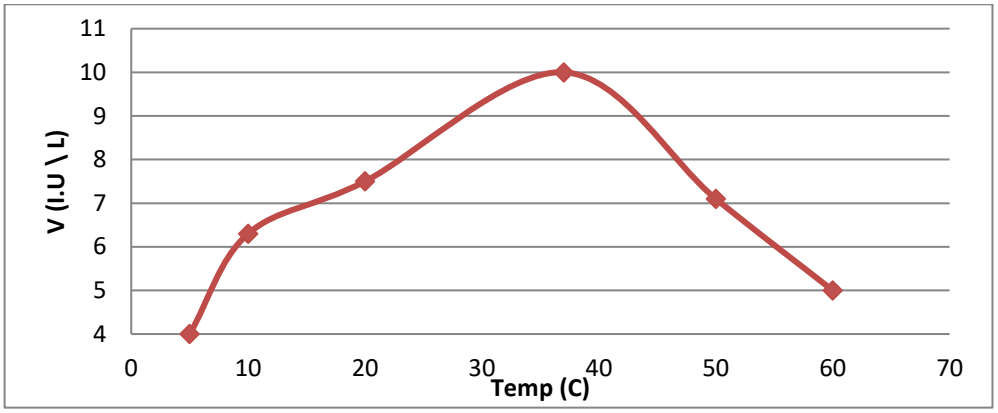


Figure (8) represents the effect of temperature on the reaction rate of the isomer (AST) (I) at a concentration of (80 mmol.dm^{-3}) and the optimum temperature for the rate.

Table (9) shows the values of the activity of the AST enzyme at different temperatures at a concentration of (166.5mmol.dm^{-3}) for the isomer (I) purified from the serum of patients with diabetes.

Temp.(C^0)	Activity AST (IU / L)
5	9.1
10	11.3
20	13
37	20
50	12.4
60	8.0

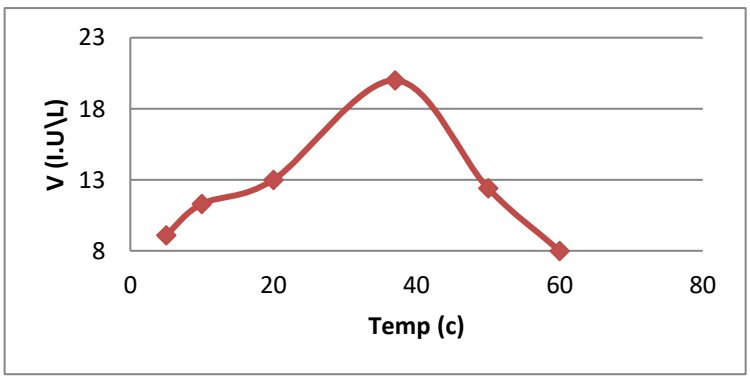


Figure (9) represents the effect of temperature on the reaction rate of the isomer (AST) (I) at a concentration of (166.5mmol.dm^{-3}) and the optimum temperature for the rate.

Table (10) shows the activity values of the AST enzyme at different temperatures at a concentration of (40mmol.dm^{-3}) for the isomer (II) purified from the serum of patients with diabetes.

Temp.(C^0)	Activity AST (IU / L)
5	5.8
10	7.4
20	11.2
37	15.3
50	9.5
60	4.7

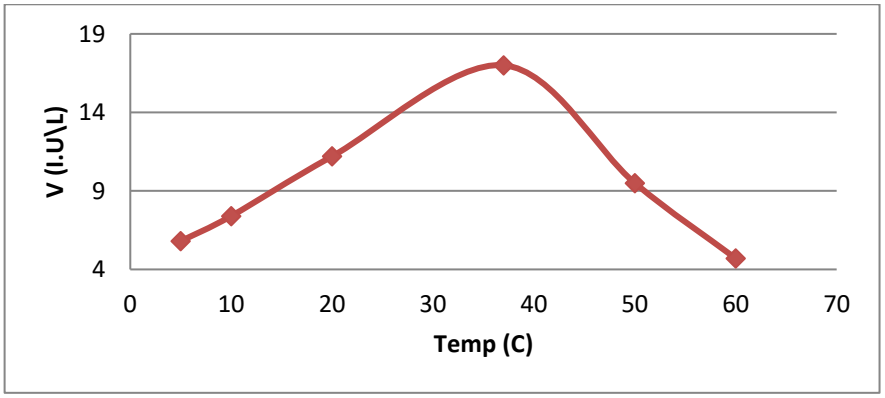


Figure (10) represents the effect of temperature on the reaction rate of the isomer (AST) (II) at a concentration of (40mmol.dm^{-3}) and the optimum temperature for the rate.

Table (11) shows the activity values of the AST enzyme at different temperatures at a concentration of (80mmol.dm^{-3}) for the isomer (II) purified from the serum of patients with diabetes.

Temp.(C^0)	Activity AST (IU / L)
5	8
10	8.7
20	9.2
37	12
50	5.3
60	3.5

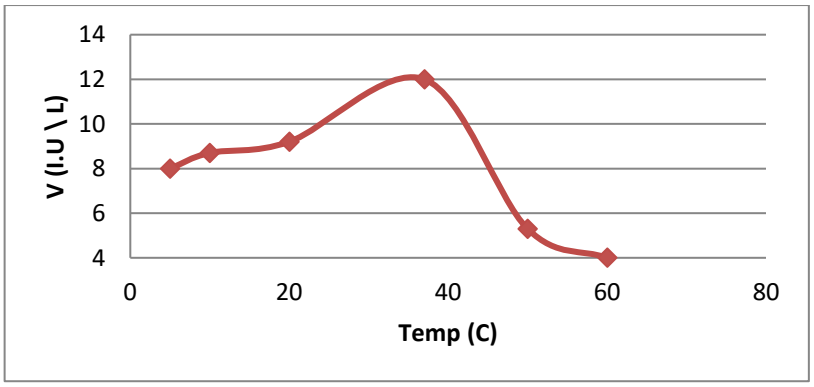


Figure (11) represents the effect of temperature on the reaction rate of the isoenzyme (AST) (II) at a concentration of (80mmol.dm^{-3}) and the optimum temperature for the rate.

Table (12) shows the activity values of the AST enzyme at different temperatures at a concentration of (166.5mmol.dm^{-3}) for the isoenzyme (II) purified from the serum of patients with diabetes.

Temp.(C^0)	Activity AST (IU / L)
5	7.7
10	12.0
20	12.6
37	22
50	6.0
60	5.1

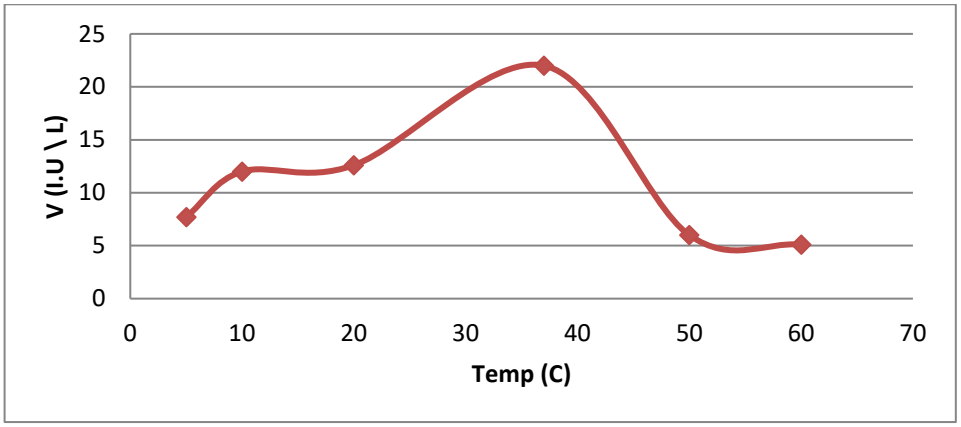


Figure (12) represents the effect of temperature on the reaction rate of the isomer (AST) (II) at a concentration of (166.5 mmol.dm^{-3}) and the optimum temperature for the rate.

b. The effect of temperature on the maximum speed (Vmax)

The relationship between the logarithm of the maximum speed of the symmetries (I, II) against the inverse of the absolute temperature was studied, which gives a straight line as in Figure (19) and which follows the following Arrhenius equation:

$$K = A\, e^{-E / RT} \quad \text{.....(17)}$$

Where:

E = activation energy.

A = Arrhenius constant.

R = gas constant of 1.987 kcal. K^{-1} mol^{-1}.

T = absolute temperature.

Each isotope is subject to the Arrhenius equation up to the optimum (37)˚C. The activation energy (Ea) was also calculated from the slope of the straight line in the above figure for the logarithm (Vmax) versus 1/T X 10^{-3}, which is represented by the equation:

$$\text{Slope} = \frac{-Ea}{2.303R}$$

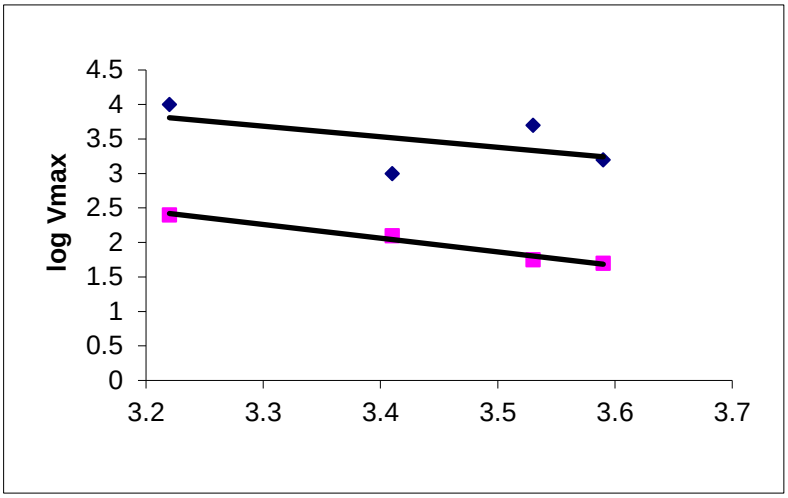
The intercept represents the logarithm of the Arrhenius constant. The amount of the effect of temperature is determined by the temperature coefficient, which is defined as the ratio between the reaction rate at the reaction temperature T+10 and its rate at T. It is symbolised by (Q_{10}). The coefficient increases the reaction rate with a ten-degree Celsius increase in temperature. Its value was calculated from the equation.

$$Ea = \frac{2.303RT2.T1\log Q10}{10}$$

Table (13) shows the values of activation energies (Ea) and the thermal coefficient Q_{10} for the isomers (I, II). From observing the table, we find that the values of Q_{10} match the fact that the thermal coefficient Q10 for enzymatic reactions lies between (2-1), i.e., within the range of enzymatic reactions [19].

Table (13) shows the values of (Ea) and (Q_{10}) for the isomers (AST) (I, II) extracted from Figure (19)

Enzymes	Ea (Cal/mol	Q_{10}
Isoenzyme I	7263	1.5
Isoenzyme II	1072	1.9



(1\T x 10^{-3}) K^{-1}

Figure (13) The linear relationship between the maximum speed logarithm versus the absolute temperature's reciprocal for the AST symmetries (I, II).

Kinetics of the reaction order of the aminotransferase (AST) isomers (I, II) purified from the serum of patients with diabetes. Figures (14) and (15) show the relationship between the reaction time and the activity of Aspartic acid at a temperature of (37) ˚C.

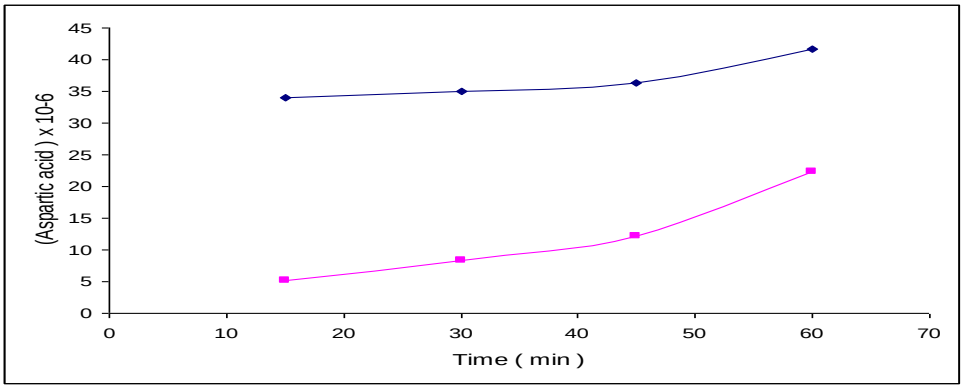


Figure (14) represents the kinetics of the association of AST isomers (I, II) at different times.

The values of Tables (4, 3, 2, and 1) represent the activity values of the AST enzyme analogues with different concentrations of aspartic acid (the base material) purified from the serum of diabetic patients and healthy people, respectively. It is noted from Figures (6, 5, 4, 3) that the speed is directly proportional to the concentration to the optimum concentration of the base material (166.5 mmol.dm^{-3}), and this gives a linear relationship to which the equation applies:

$$V = \frac{Vmax\,[S]}{km+[S]}$$

Km >> [S] is used for both analogues and then the concentration of the basic substance is removed from the denominator because it is small compared to Km, as shown in Table (5). Therefore, the above equation can be reduced and written in the following form: V = K [S]

Where: $K = \frac{Vmax}{Km}$

K represents the rate constant, and its unit, according to the equation above, is (min^{-1}), which represents the unit of the constant when the reaction is of the first order. Thus, the first-order equation is in the following form:

$$\log [S] = - \frac{K\,t + \log [So]}{2.303}$$

When drawing the relationship between [S] log versus time as in Figure (15), the slope of the resulting straight line is equal to (-K / 2.303), from which the value of the velocity constant (k) is calculated for the symmetries (I, II). As for the intersection of the straight line with the point on the vertical axis, it represents log [$S_{\circ}$] [24,26].

Table (14) shows the values of the velocity constant extracted from the first-order equation.

Isoenzyme	k(min^{-1})
I	0.0172
II	0.0174

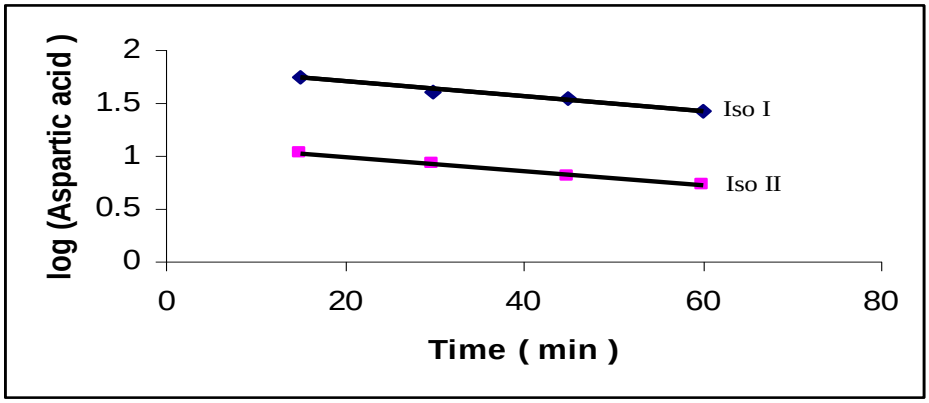


Figure (15) represents a logarithm graph of the concentration of the base material versus time from the first-order velocity equation for the isotopes (I, II) at a temperature of (37) °C.

2. Thermodynamic studies of the binding of the substrate to the amino group transfer enzyme (AST) isomers (I, II).

Thermodynamic parameters of the standard state

The values of the thermodynamic functions (ΔS, ΔG, ΔH) were calculated from the following equations:

Where the value of Gibbs's free energy (ΔG) was calculated from the equation:

$$\Delta G^{\circ} = - RT \ln Ka$$

Where:

Ka = equilibrium constant

R = gas constant, which is 1.987cal. mol^{-1}. k^{-1}

T = absolute temperature

The value of the enthalpy change of the standard state ΔHo was calculated from the Vant Hoffs

equation as shown in Figure (22), where the values of ln Ka were plotted against the reciprocal of temperature in Kelvin based on the following equation:

$$\text{Ln Ka} = \frac{-\Delta H}{RT} + c$$

Where:

ΔH_o = enthalpy change.

R = universal gas constant (1.987cal. mol^{-1}. k^{-1}).

The value of H can be calculated from the slope of the straight line of the Van't Hoff equation, which is equal to ($R / -\Delta H_o$).

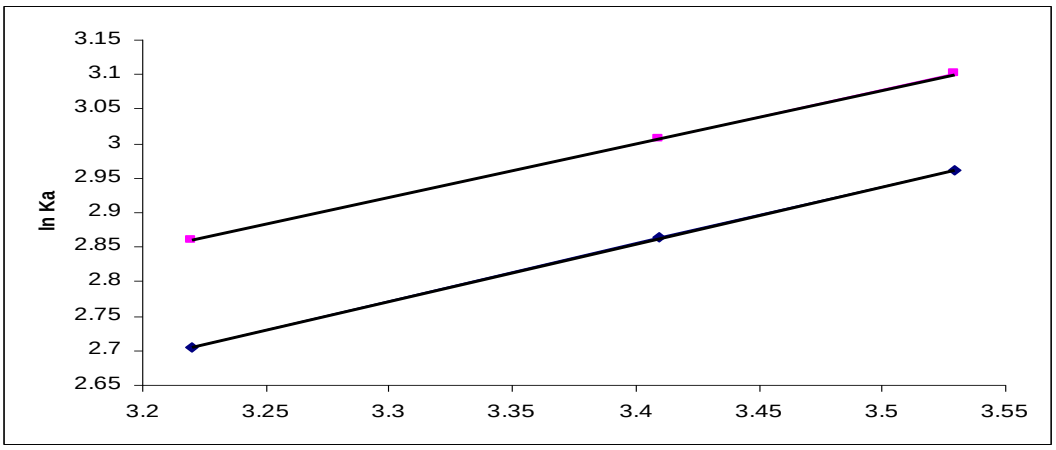


Figure (16) represents the van't Hoff diagram of the relationship between the basic substance and the symmetries (I, II).

As for ΔS, it is calculated from the following equation:

$\Delta S^{\circ} = (\Delta H^{\circ} - \Delta G^{\circ}) / T$

Thermodynamic parameters of the transition state

The thermodynamic parameters of the transition state were studied using the following equations:

$$\Delta H^* = Ea - RT$$

$$\text{Log } V_{max} = \log A - Ea / 2.303\ RT$$

:Where

A = Arrhenius constant

Ea = activation energy

The graph resulting from plotting Vmax Log versus 1/T gives a straight line with a slope equal to the value of (– Ea / 2.303 R), while the value of ΔH* is calculated from the following equation:

$$\Delta H^* = Ea - RT$$

ΔG* is calculated from the equation:

$\Delta G^* = -RT \log V_{max} + RT \ln (KT / h)$

Where:

K = Boltzmann constant and equals 1.3806 X 10-23J.deg^{-1}.

h = Planck constant and equals 6.626 X 10-34J.S^{-1}.

The change in entropy of the transition state (ΔS*) can be calculated from the equation:

$\Delta S^{*} = (\Delta H^{*} - \Delta G^{*}) / T$

Table (15) shows the values of the thermodynamic functions for the standard and transition states of the bonding of the base material with the isomers (I, II) at a temperature of (37) ˚C.

Isoenzyme	ΔH cal . mol^{-1}	ΔG cal. mol^{-1}	ΔS cal .deg^{-} . mol^{-1}	ΔH* cal . mol^{-}	ΔG^{*} cal. mol^{-1}	ΔS^{*} cal.deg^{-1}. mol^{-1}
I	-1529.9	-1664.78	134.79	7804.03	17875.64	-32.49
II	-1629.3	-1764.43	135.09	8464.03	17958.60	-30.62

Table (15) shows the values of (ΔS, ΔG, ΔH) for the first and second isomers in the standard state and at a temperature of (37) ˚C. From observing the values mentioned in the table above, we find that the value of ΔH for the symmetries is negative, which means that the reaction is exothermic. In contrast, the value of ΔG0 is negative, meaning that the reaction is spontaneous and that it is equipped with appropriate energy towards the formation of the complex (AST – Aspartic acid), ΔS is positive, which means that the process is not organized (i.e. random) and that these three functions depend on the initial state and the final state of the association (of the system) and do not depend on the mechanism and direction of the reaction (as they are state functions). The results of Table (15) also show the difference in the values of the thermodynamic functions (ΔG, ΔS) for the standard and transition states, as we find a difference in the sign of the value of ΔG and its transformation from a negative value in the standard state to a positive value in the transition state and vice versa for ΔS, which indicates that the complex formed from the association of the basic material with the symmetries is formed spontaneously in the basic state and the negative value of (ΔS*) makes the complex more regular than the base case since ΔS > ΔS* [26], [27].

4. Conclusions

- Increased activity of (AST) in the serum of patients with diabetes can be relied upon in the diagnosis of diabetes
- The difference in the values of the kinetic constants of the enzyme analogues, such as concentration and pH, confirms the variation of these analogues
- Many general equations can be used to find the reaction order of the enzyme analogues that transfer the amino group
- We conclude from thermodynamic studies that the driving force of the binding reactions depends on the function of the change in entropy

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