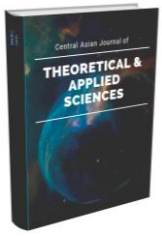

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Article

Biodynamic and Thermodynamic Study of Alanine Aminopeptide (AAP) Partially Purified from the Urine of Patients with Chronic Renal Injury

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Abstract: Alanine aminotransferase (AAP) activity was measured in the urine of healthy individuals, with an activity level of 7.49 ≤ 2.76 IU/L. In infected individuals, the activity level was 24.83 ≤ 9.66 IU/L. The results showed elevated AAP activity in the urine of 97% of patients compared to healthy individuals. AAP was purified from the urine of patients with renal failure using gel filtration chromatography with Sephadex G-50 filtration gel. AAP was separated from the urine of patients with renal failure using ion-exchange chromatography with DEAE-Sephadex A-50 resin. It was observed that the isoform obeys the Arrhenius equation up to 37 m^{-1}, and Ea and Q10 were determined. A thermodynamic study was performed on the binding of the substrate (alanine-4-naturoanilide) to the alanine aminopeptide (AAP) isoforms. Using the van't Hoff and Arrhenius equations, the thermodynamic coefficients for the standard state (ΔSO, ΔGO, ΔHO) and the transition state (ΔS*, ΔG*, ΔH*) were determined.

Keywords: Alanine Aminopeptidase (AAP), Renal Failure, Urinary Biomarker, Enzyme Kinetics, Thermodynamic Parameters.

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1. Introduction

Kidney failure is a common chronic disease worldwide, characterized by a gradual or sudden decline in the kidneys' ability to perform their vital functions, primarily filtering waste products from the blood and regulating fluid and electrolyte balance. This disease is closely linked to modern diseases such as diabetes and hypertension, making it an increasing health challenge that impacts quality of life and increases morbidity and mortality rates. The danger of kidney failure lies in its silent nature in the early stages, necessitating a focus on early detection and health awareness to reduce its complications and improve treatment outcomes [1]. Enzymes found in human urine have become important in diagnosing many diseases, including kidney failure, as they serve as indicators of kidney tissue damage.

Alanine aminopeptidase (AAP)

This enzyme is widely distributed in kidney tissue [2], in addition to being present in blood serum [3], the mucous membrane of the small intestine [4], the liver, pancreas [5], and the placenta and prostate gland in humans [6]. The enzyme is not limited to human organs; it is also found in the tissues of some animals, with the kidney being one of the richest sources. Its activity in the kidney is approximately 10-15 times higher than in other organs, thus making the kidney the primary source of the enzyme [7].

Purification of Alanine Aminopeptidase (AAP)

Several methods have been used to purify the enzyme from different sources, employing various techniques including dialysis and different chromatographic methods. Human kidney AAP was purified after homogenization using electrophoresis and centrifugation [8]. Mattenheimer et al. also succeeded in... (1986) purified the enzyme from human and animal urine using first membrane sorting and then gel filtration with Sephadex G-25 [9]. Jung et al. (1980) also purified the enzyme from human urine using gel filtration, but with Sephadex G-50 [10]. Kinetic Properties of Alanine Aminopeptidase (AAP)

The Michaelis-Menten constant (Km) values differ between the two enzyme isoforms purified from healthy urine. The first isoform gives a low value of (4-10 × 1.3) mol/L, while the second gives a high value of (3-10 × 1.86) mol/L [9]. Temperature affects the rate of the enzyme reaction; an increase in temperature increases enzyme activity, provided that the temperature does not reach a level that leads to enzyme denaturation. (Denaturation) [11]. It was found that when urine or the osmotic fraction is stored at 4°C, the enzyme maintains its activity for six days. It was also found that when urine is stored at -12°C, the enzyme loses its activity within one day, while the osmotic fraction can be stored at this temperature without losing its activity for more than a week [10]. Thermodynamics of Alanine Aminopeptide (AAP)

Thermodynamics studies the properties of groups under equilibrium conditions and does not concern itself with the effects of time. Through it, we obtain precise relationships between different measurements and provide the basis for predicting the effects of heat and pressure, focusing on chemical equilibrium. In a chemical reaction, the substrate requires a large amount of energy to reach the transition state, and the energy required to form the product is reduced by the presence of the enzyme. As with other catalysts, the enzyme AAP does not change the position of the chemical equilibrium of the reaction. That is, the reaction proceeds in the same direction in the presence of AAP, but at a faster rate. However, in the absence of AAP (AAP) can cause spontaneous, uncatalyzed reactions that lead to different results [12], [13].

2. Materials and Methods

Specimens

Fifteen (15) urine samples were collected from individuals with renal failure, and ten (10) urine samples were collected from healthy individuals after confirming they were not affected by the disease. Ten centimeters of urine were collected in a clean, dry, single-use tube. Enzyme activity was measured immediately after collection; the sample was not stored for the following day.

Procedure:

Alanine aminopeptide (AAP) activity is measured as follows:

1. Mix 1.0 ml of buffer solution (Tris-HCl) at a concentration of 50 mmol/L and pH 7.8 with 0.2 ml of the sample. Incubate the tube at 37°C for 10 minutes. Then, add 0.1 ml of base solution (alanine-4-nitroaniline hydrochloride) at a concentration of 2 mmol/L to initiate the reaction.
2. After adding the base solution, mix the tube thoroughly and measure the change in absorbance over 2 minutes at a wavelength of 410 nm.
3. Determine the enzyme activity by comparing the absorbance to a standard graph for different concentrations of 4-nitroaniline. Separation and Purification of Isomorphic Enzymes: AAP was purified from the urine of healthy individuals and patients with renal failure.

AAP was purified from urine according to the method of Jung and Scholz (1980) [10]. This was done to remove inhibitors that limit its activity, using two steps [14]:

a) Gel Filtration:

The enzyme was purified from urine using a Sephadex G-50 gel filtration column, which operates based on molecular weight differences. It was prepared by dissolving 2 grams of Sephadex G-50 powder in 200 cm^3 of Tris buffer solution at a concentration of 0.05 mol/L and pH 7.8. The solution was left to stand for 24-48 hours at 4°C, during which the Tris buffer solution was changed several times by decanting to remove fine particles. Its presence leads to blockage of the small pores, thus reducing the flow rate of the leached solution through the column.

Procedure:

1. A glass column with a diameter of 1.5 cm and a length of 20 cm is used. A small amount of glass wool is placed at the end of the column to prevent the gel particles from escaping. The gel filtrate solution is poured into the column slowly and uniformly to prevent air bubbles from forming until the gel reaches a height of 8 cm. The gel column is then washed with sufficient quantities of Tris buffer solution at a pH of 7.8 until a flow rate of 2.5 cm^3 per minute is achieved.
2. Five cm^3 of urine is added slowly on top of the surface of the filtrate gel.
3. The separation process begins using the Tris buffer solution. 25 cm^3 of Tris buffer solution is added, and six tubes of leached material, each containing 5 cm^3, are collected at a temperature of 4°C. B - Using a negative ion-exchange chromatography column, where the resin (DEAE – Sephadex A – 50) was used to separate the alanine aminopeptide (AAP) isomers from the urine of healthy individuals and patients with (renal insufficiency). Solutions used in this method: DEAE-Sephadex A-50 gel suspension, Tris buffer solution (0.05 mol/L), sodium chloride solution (0.1 mol/L), sodium chloride solution (0.2 mol/L), sodium chloride solution (0.3 mol/L).

Procedure:

1. A glass column 1.5 cm in diameter and 20 cm long is used. A small amount of glass wool is placed at the end of the column to prevent the gel particles from escaping. The gel suspension is poured into the column slowly and uniformly to prevent air bubbles from forming until the gel reaches a height of 8 cm. The gel column is then washed with Tris buffer solution at a pH of 7.8 until a flow rate of 24 cm^3/24 min is achieved.
2. Add 2 ml of urine slowly to the surface of the gel and allow it to remain in the column for 3-5 minutes to absorb it.
3. Separate using 22 ml of Tris buffer solution. Collect 12 tubes of 2 ml each containing the oozing fraction. Then, separate using Tris buffer solution containing varying concentrations of sodium chloride. The process is as follows:

a) Add 12 ml of Tris buffer solution containing 0.1 mol/L sodium chloride solution to the gel column and collect 6 tubes of 2 ml each containing the oozing fraction.
b) Add 12 ml of a second Tris buffer solution containing 0.2 mol/L sodium chloride solution to the gel column and collect 6 tubes of 2 ml each containing the oozing fraction.
c) Add 12 ml of Tris buffer solution III, containing 0.3 mol/L sodium chloride solution, to the gel column and collect 6 tubes of 2 ml each.
d) Add 24 ml of Tris buffer solution IV, containing 0.4 mol/L sodium chloride solution, and collect 12 tubes of 2 ml each.

Kinetic Studies of Partially Purified Alanine Aminopeptide (AAP) I Urine from Patients with Renal Impairment

The kinetic properties of alanine aminopeptide (AAP) isoforms were studied in the partially separated and purified amyloid fractions.

1. Effect of Base Concentration (Alanine-4-nitroanilide)

The effect of different base concentrations (Alanine-4-nitroanilide) on the reaction rate of isoform I was investigated using various concentrations of Alanine-4-nitroanilide (0.3, 0.5, 0.7, 0.9, 1.5, 2, and 2.5 mmol/L) to determine the optimum concentration of the isoforms. The optimum concentration, at which the reaction rate reaches its maximum, was calculated by plotting the relationship between the reaction rate and the base concentration.

The optimum concentration is the concentration at which the reaction rate reaches its maximum.

2. Determining the values of the Michaelis-Menten constant (Km)

To determine the values of the constant (Km) for the base material used (alanine-4-nii) The same method was used for the Truanlid method, and the values of the constant (Km) were obtained using the following methods for plotting the graphs:

a) The Lenover-Berke method, which relates the inverse values of both the rate and the concentration of the substrate (1/V Vs. 1/[S]).
b) The Hans Wolff method (S/V Vs. [S]).

3. Effect of pH

The effect of pH on the rate of the reaction of isomer (I) was studied. Different pH values of the Tris buffer solution (6.6, 7.0, 7.4, 7.8, 8.0, 8.6) were used in the presence of a substrate solution with a concentration of (2) mmol/L. The reaction was carried out in a water bath at a temperature of (37)°C, and the tubes were incubated for ten minutes.

Effect of pH on the Michaelis constant (km) of the purified alanine aminopeptide (AAP) isoform from the urine of patients with renal impairment:

The effect of pH on the Michaelis constant was investigated using different pH values (6.6, 7.0, 7.4, 7.8, 8.0, 8.6) of the Tris buffer solution, in the presence of different concentrations of alanine-4-nitroanilide (0.5, 0.9, 2) mmol/L. The reaction was carried out in a water bath at 37°C for 10 minutes. The Lenover-Berck equation was used to calculate the Michaelis constant. The enzyme activity of the isoforms in the exudates was measured according to section (1), and the relationship between the obtained pkm and pH was then plotted.

4. Effect of Temperature

This experiment was conducted to study the effect of different temperatures on the reaction rate of the enzyme isomer Alanine Aminopeptide (AAP) (I). The reaction was carried out at different temperatures (5, 10, 20, 37, 50, and 60) °C, in the presence of a buffer solution of Tris pH (7.8) and an optimal concentration of 2 mM/L of the substrate solution (alanine-4-nitroanilide).

Thermodynamic Studies of Isomers

The thermodynamic coefficients for the standard state and the transition state were calculated using the Vant Hoff and Arrhenius equations, respectively.

3. Results and Discussion

Measurement of Alanine Aminopeptide (AAP) Activity in the Urine of Healthy Individuals and Patients with Renal Impairment

Twenty-five (25) samples were tested, fifteen (15) of which were from patients with renal impairment, with enzyme activity levels ranging from 12 to 52 IU/L. Ten (10) samples were from healthy individuals, with enzyme activity levels ranging from 4 to 14 IU/L. A comparison of AAP activity levels in the urine of patients with renal impairment with those of healthy individuals revealed a higher level in the patients than in the healthy individuals [10]. As in Figure 1.

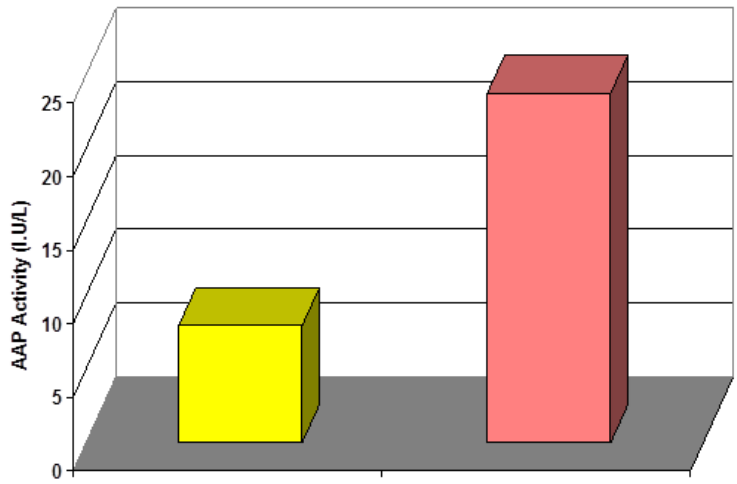

Figure 1. AAP activity values in the urine of healthy and sick individuals.

Separation and Purification of Alanine Aminopeptide (AAP) Isomers in the Urine of Healthy Individuals and Patients with Renal Insufficiency:

Membrane separation and gel filtration are among the methods used to purify enzymes [15]. Measurements of enzyme activity before and after separation showed an increase in activity. Enzyme activity was observed after separation by membrane separation and then by gel filtration using Sephadex G-25 gel [14]. When separation was performed using ion-exchange chromatography with DEAE-Sephadex A-50, enzyme activity increased even further. This is attributed to the removal of all inhibitors that reduce enzyme activity. Two isoforms of the enzyme are shown, as illustrated in Figure 2.

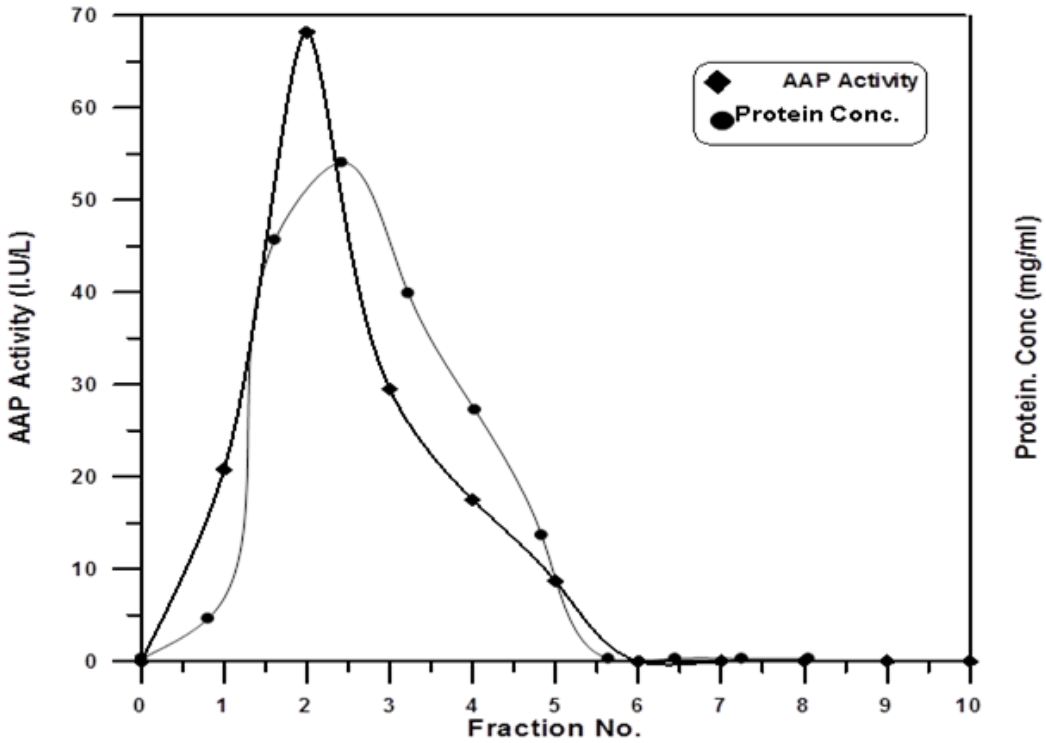

Figure 2. Partial purification of (AAP) from the urine of patients with (renal failure) using gel filtration chromatography.

Kinetic Studies of AAP Analogs

1. Optimal Concentration of Alanine-4-Nitroanilide

Figure 3 illustrates the effect of Alanine-4-Nitroanilide concentration on the rate of the enzymatic reaction of the two analogs. The rate increases with increasing Alanine-4-Nitroanilide concentration until it reaches a maximum rate at 2 mmol/L. The reaction rate then begins to decrease at higher concentrations of Alanine-4-Nitroanilide due to inhibition. When the concentrations of Alanine-4-Nitroanilide that inhibited the reaction rate of analogs I and II were removed, and the relationship between Alanine-4-Nitroanilide concentrations and the reaction rate of analogs I and II were redrawn, and the logarithmic statistical equation was used to plot the optimal curve for computational purposes in kinetic studies (as shown in Figure (4), the hyperbolic curve of the analogs was found. Enzymes (I and II). To confirm the kinetic equation used, the concentrations of the substrate (alanine-4-nitroanilide) that give 75% and 25% of the maximum speed were calculated, and then their percentage was calculated as follows[16]:

$$\frac{[Alanine-4-nitroanilide]_{0.75}}{[Alanine-4-nitroanilide]_{0.25}}$$

The ratio of the base material (alanine-4-nitroanilide) was for isomer I (3.2) and isomer II.(4.0).

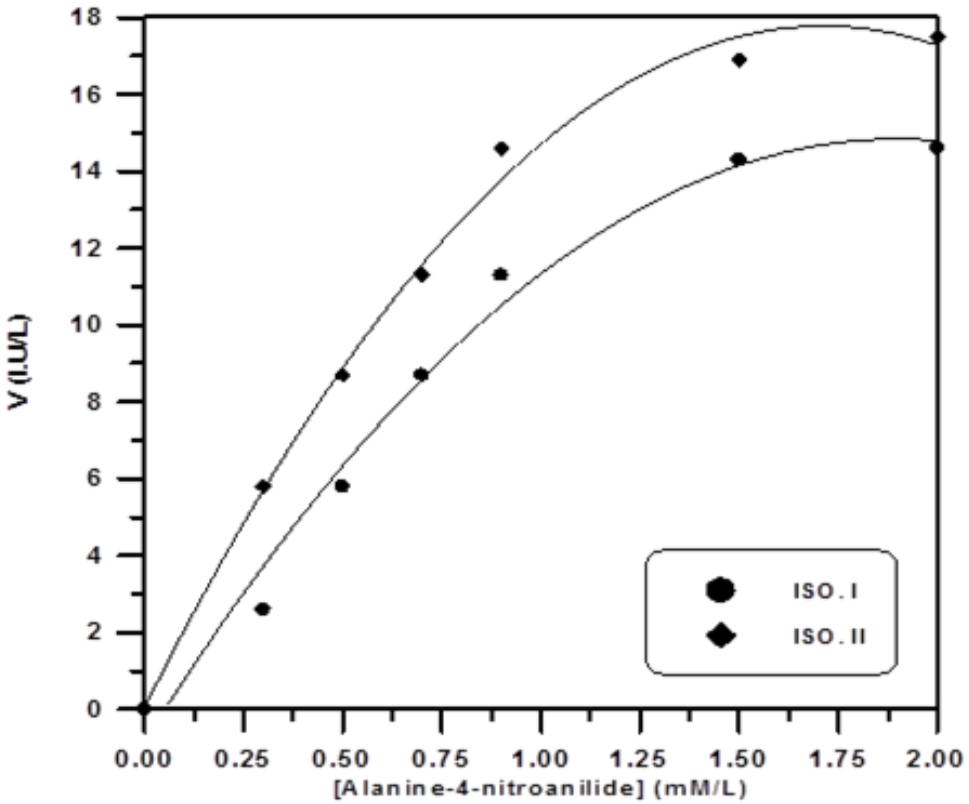

Figure 4. The relationship between different concentrations of (alanine-4-nitroanilide) mmol/L and the reaction rate of the purified isomers I and II from the urine of patients with (renal failure).

Determining the values of the Michaelis-Menten constant (Km)

The Lenover-Berke and Hans Wolff equations were followed to calculate the values of the Michaelis-Menten constant (Km) for the (AAP) (I) isomers in the urine of healthy individuals and patients with renal failure. The value of the Michaelis constant for isomer I in the urine of healthy individuals was calculated to be (3-10 × 1.3 mol/L) according to the

Lenover-Berke equation and (3-10 × 1.2 mol/L) according to the Hans Wolff equation, as shown in Figures (5) and (6). In the urine of patients with renal failure, it was (3-10 × 10 mol/L) according to the Lenover-Berke equation and (3-10 × 9 mol/L) according to the Hans Wolff equation, as shown in Figures (7) and (8).

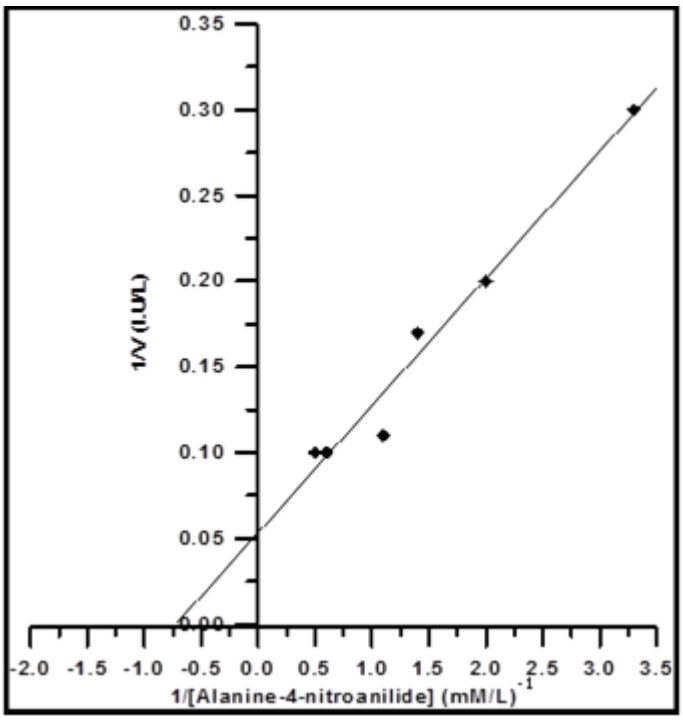

Figure 5. Linover-Birke diagram for calculating the Michaelis-Menten constant for isomer I purified from the urine of healthy individuals.

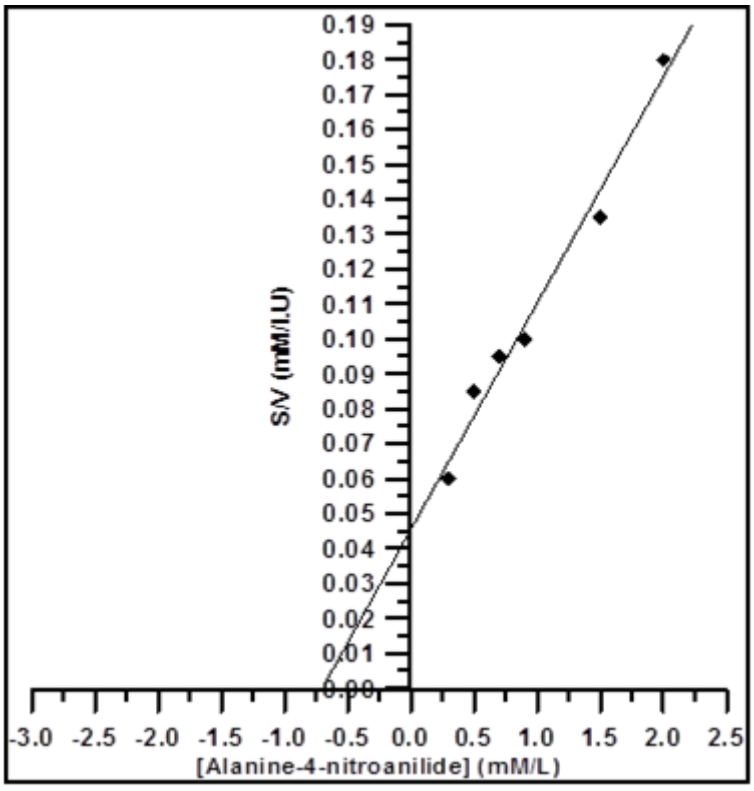

Figure 6. Hutter-Wolff diagram for calculating the Michaelis-Menten constant for the purified isomer I from the urine of healthy individuals.

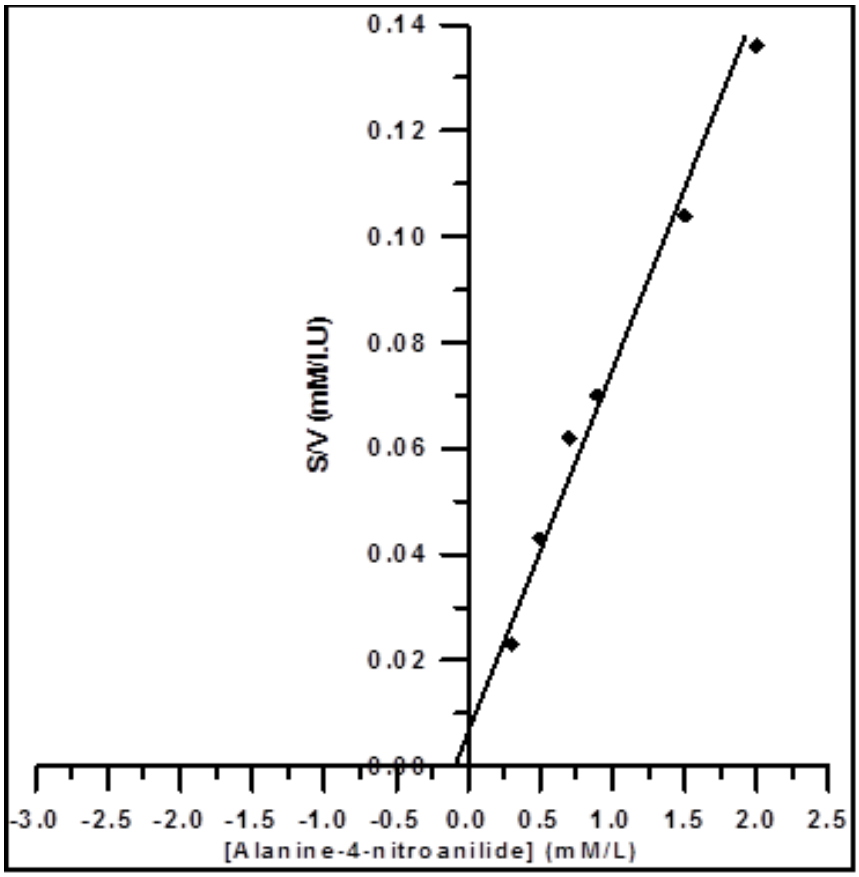

Figure 7. Hutter-Wolff diagram for calculating the Michaelis-Menten constant for the purified isomer I from the urine of patients with renal insufficiency.

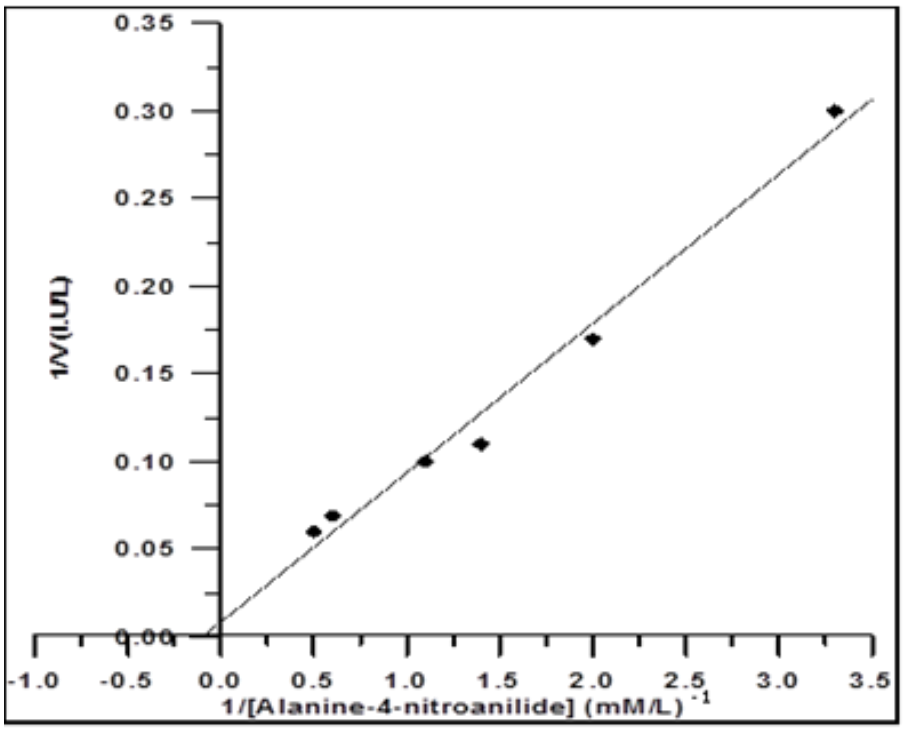

Figure 8. Linover-Birk diagram for calculating the Michaelis-Menten constant for the purified isomer I from the urine of patients with renal insufficiency.

Table 2. Michaelis-Menten constant (Km) values for (AAP) I and II isomers partially purified from the urine of healthy individuals and patients with renal impairment, based on the Lenover-Berke and Hans Wolff equations.

Enzyme	Substrate	Km (mole/liter)			
		1/v Vs. 1/[S]		[S]/v Vs.[s]	
		Normal	Injured	Normal	Injured
Isoenzyme I	Alanine-4-nitroanilide	1.3×10^{-3}	10×10^{-3}	1.2×10^{-3}	9×10^{-3}
Isoenzyme II	Alanine-4-nitroanilide	6.3×10^{-3}	2.1×10^{-3}	5×10^{-3}	1.1×10^{-3}

Effect of pH on the reaction rate of alanine aminopeptide (AAP) analogs and on the Michaelis-Menten constant

General relationship

It is evident that pH affects the rate of enzymatic reactions. When plotting the relationship between different pH levels (6.6, 7.0, 7.4, 7.8, 8.0, 8.6) and the enzymatic reaction rate of analog I, it was found that the reaction rate of both analogs increases with increasing pH until it reaches the maximum reaction rate at pH 7.8 for analog I. After that, the reaction rate decreases with increasing pH, as shown in Figure 9.

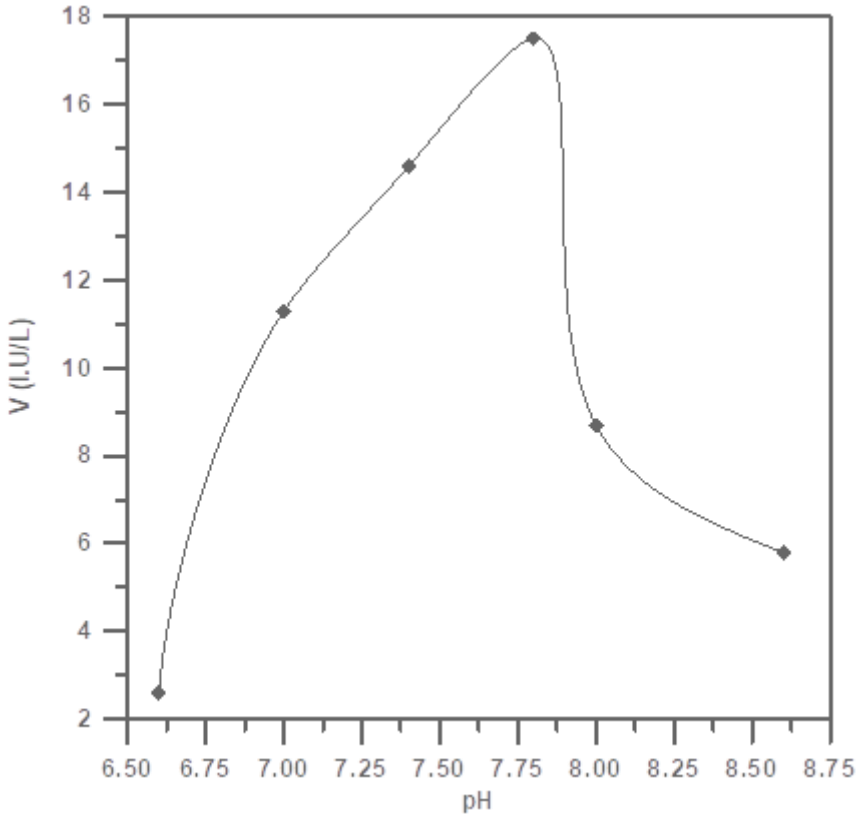

Figure 9. The effect of pH on the reaction rate of the purified isomer I from the urine of patients with renal failure.

The Effect of pH on the Michaelis-Menten Constant (Km)

The effect of pH changes on the value of the Km constant was studied, and its value was calculated using the Lineweaver-Burk equation. The Michaelis-Menten constant is affected by pH. The pKm values, representing the pH values of the amino acids located at the active sites of the I-isomer, were determined by plotting the relationship between pKm

and pH. The pKm value for the I-isomer, as shown in Figure 10, was found to be 7.0, corresponding to the amino acid histidine.

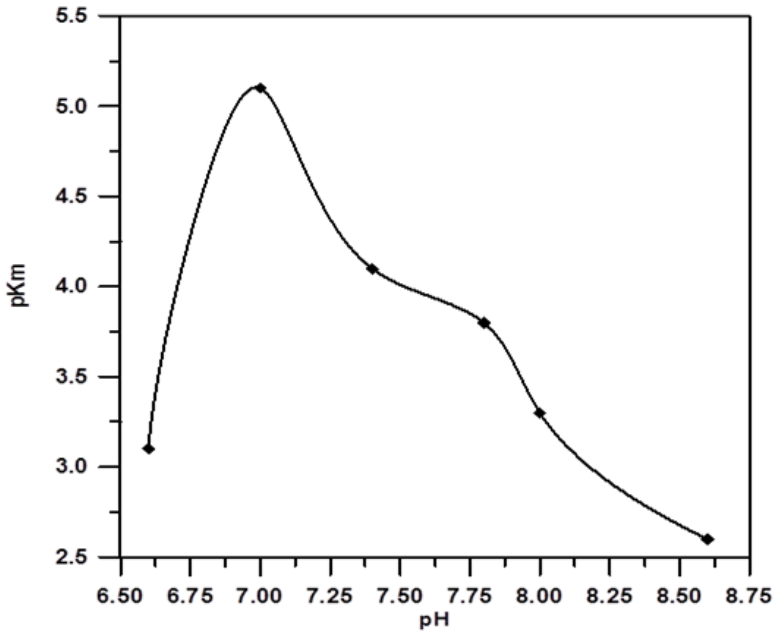

Figure 10. Effect of pH on the Michaelis-Menten constant of the purified isomer I from the urine of patients with renal insufficiency.

Effect of temperature on the reaction rate of alanine aminopeptides (AAPs) and on the maximum reaction rate (Vmax)

General relationship

Temperature affects the ionization state of the groups on the enzyme surface and on (alanine-4-nitroanilide). Figure 11 shows the effect of temperature on the reaction rate of alanine aminopeptides (AAPs). The reaction rate increases with increasing temperature until it reaches the maximum reaction rate at a temperature of 37°C, after which the reaction rate begins to decrease.

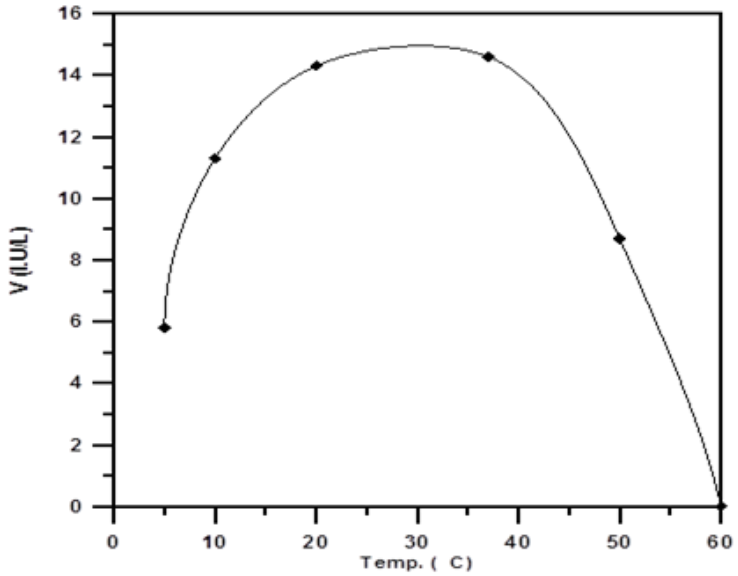

Figure 11. Effect of temperature on the rate of a symmetric reaction (AAP) I using different temperatures (5, 10, 20, 37, 50, 60)°C with all conditions remaining at their optimal state.

Effect of temperature on maximum velocity Vmax

The relationship between the logarithm of maximum velocity (V_{max}) of symmetry (I) and the inverse of absolute temperature, which gives a straight line as in Figure (12), was studied, following the Arrhenius equation [17]:

$$\text{Ln K} = \frac{-Ea}{RT} + \text{Constant}$$

The symmetry obeys Arrhenius' equation up to 37 °C. The activation energy of the reaction (E_a) was calculated from the slope of the graph in Figure (12) of the logarithm (Vmax) against $1/T \geq 10^{-3}$, represented by the following equation:

$$\text{Log K} = \frac{-Ea}{2.3R} \cdot \frac{1}{T} + \log A$$

Therefore, the slope of the graph $\frac{-Ea}{2.3R}$ =

The magnitude of the effect of temperature is determined by the temperature coefficient, which is defined as the ratio between the rate of reaction at the reaction temperature t+10 and its rate at temperature t, and is denoted by (Q_{10}). It is the coefficient that increases the rate of reaction with increasing temperatures (10)°C.

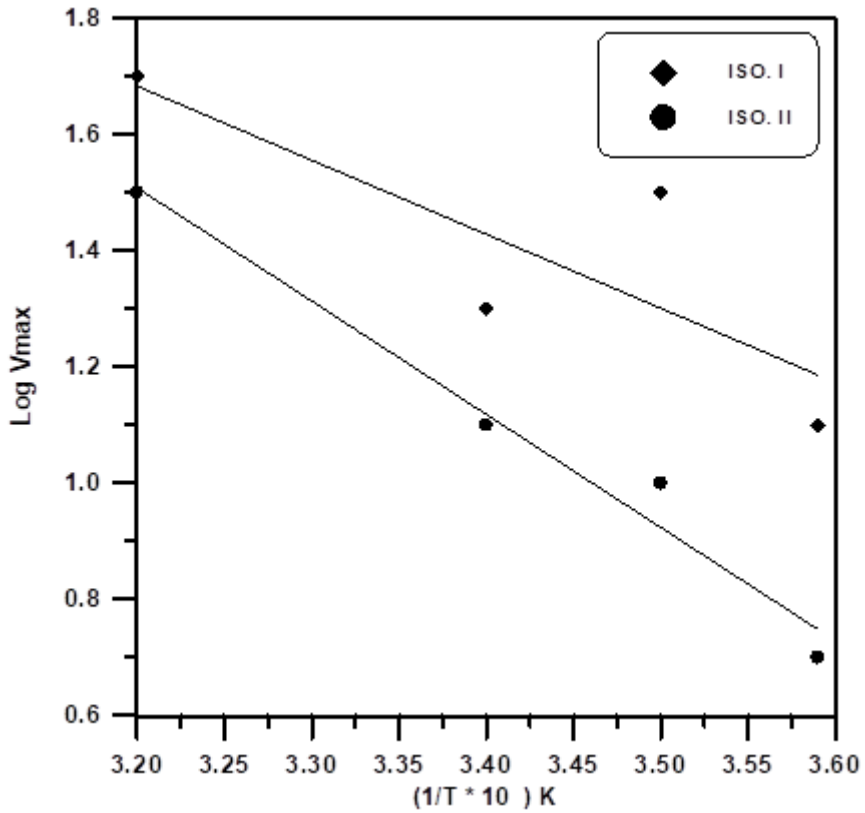

Figure 12. Arrhenius diagram of the inverse effect of absolute temperature on the logarithm of maximum velocity for AAP symmetries I and II.

Table 2. Values of (E_a) and (Q_{10}) for AAP symmetries I and II.

Enzymes	E_a (Cal)	Q_{10}
Isoenzyme I	30087	1.0004
Isoenzyme II	38294	1.0005

Table 2 shows the activated energy values Ea and the Q10 values for the two symmetries, and Q10 was determined by the equation:

$$E_a = \frac{2.3RT_2.T_1 \log Q_{10}}{10}$$

From observing the table, we find that the value of Q_{10} corresponds to the fact that the values of the temperature coefficient Q_{10} for enzyme reactions fall between (1-2), i.e., they fall within the range of enzyme reactions [16].

Thermodynamic studies of the (alanine-4-nitroanilide) association of alanine aminopeptide (AAP) analogs I and II

1- Thermodynamic parameters of the standard case

The effect of temperature on the affinity constant (K_a) was studied using Vant Hoff's law, as shown in Figure (13). The values of lnKa were plotted against the reciprocal of the temperature in Kelvin, based on the following equation:

$\ln K_a = \Delta S°/R - \Delta H^o /RT$

Where:

ΔS^0 = Change in entropy of the standard state.

ΔH^0 = Change in enthalpy of the standard state.

R = Universal gas constant (8.314 $J \cdot K^-$).

The values of K_a are calculated from the Scatchard diagram at different temperatures. The value of ΔH can be calculated from the slope of the straight line of the van't Hoff equation. Gibbs free energy is calculated from equation [18]:

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

As for Δ S° it is calculated from the following equation:

$\Delta S° = (\Delta H^o - \Delta G^o)/T$

Table (3) shows the values of (ΔS°, ΔG°, ΔH°) for isomers (I and II) under standard conditions at a temperature of (37)°C.

From observing the values mentioned in the table above, we find that the value of ΔH° for isomers I and II is positive. This means that the reaction is endothermic [19]. The negative value of ΔG° indicates that the reaction is spontaneous and that it is supplied with suitable energy for the formation of the (AAP-Alanine-4-nitroanilide) complex. The positive value of the standard enthalpy change can also be attributed to favorable interactions between the alenine-4-nitroanilide groups and the I and II isomers. These interactions involve non-covalent bonds that are electrostatic in nature, such as charge-charge, charge-polar, polar-polar, and hydrogen bonds [19]. These bonds contribute to the stability of the complex's twist, which is consistent with the positive value of the standard enthalpy.

Thermodynamic parameters of the transition state

Thermodynamic parameters of the transition state were studied using the Arrhenius equation, which relates the rate constant K_{+1} to the maximum reaction rate Vmax (in the biochemical equation with the reciprocal of the temperature in Kelvin), based on the following equation:

$$\ln K_{+1} = \ln A^{\frac{-E_a}{R}\ \frac{1}{T}}$$

$$\log V_{max} = \log A - E_a/2.303\ R^{\frac{1}{T}}$$

The resulting graph of Vmax log versus 1/T gives a straight line with a slope equal to the value of (-Ea/2.303 R), as shown in Figure 17. ΔH^* is calculated from the following equation:

$^*\Delta H = E_a - RT$

While Δ G* is calculated from the equation:

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

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

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

Table 3 shows the values of (ΔS*, ΔH*, Ea, ΔG*) for the two analogs in the transition state. The information we have obtained, along with all the thermokinetic data, gives us a general idea of the nature of the forces controlling the formation of the complex. From this information, we know that the complex (AAP-Alanine-4-nitroanilide) undergoes three thermokinetic stages. Stage A shows the initial energy level of (AAP-Alanine-4-nitroanilide and the two AAP analogs). Stage B shows the bonding pattern for the formation of the active complex (AAP-Alanine-4-nitroanilide). Stage C shows the formation of the reactive complex (AAP-Alanine-4-nitroanilide).

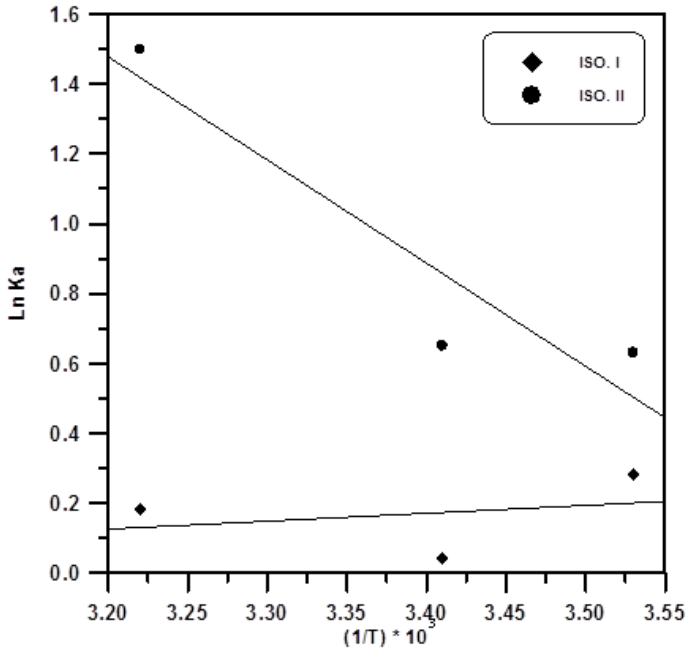

Figure 16. Van 't Hoff diagram of the relationship of the basic substance with the I and II symmetries.

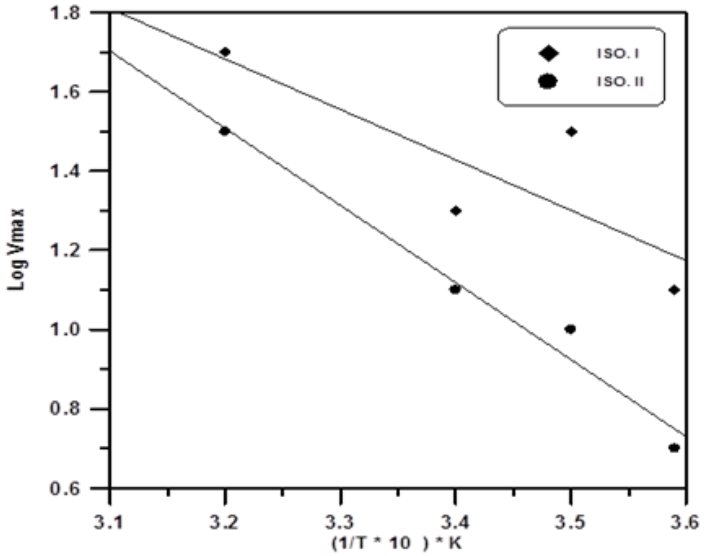

Figure 17. Arrhenius diagram of the association of the basic substance with the symmetries I and II.

Table 3. Thermodynamic parameters for the standard and transition states of (alenine-4-nitroanilide) bonding with isomers I and II at a temperature of 37 °C.

Isoenzyme	Δ H° Kj. mole^{-1}	Δ G° Kj. mole^{-1}	ΔS° J.deg^{-1} mole^{-1}	Δ H* Kj. mole^{-1}	Δ G* Kj. mole^{-1}	ΔS* J.deg^{-1} mole^{-1}	E_a KJ.mole^{-1}
I	1.5	-3.8661	4.85	29.77	-4.40	110.25	125.93
II	0.33	-0.463	1.06	37.98	-3.88	135.07	160.29

4. Conclusion

- Elevated AAP activity in the urine of patients with renal impairment can be used in the diagnosis of urinary tract diseases.
- Alanine aminotransferase (AAP) has two enzyme isomers in the urine of patients with renal impairment, with higher activity than in healthy individuals.
- The different values of the kinetic constants for isomers I and II, such as Km, optimum substrate concentration, pH, and sensitivity to the inhibitor used, confirm the variability of these isomers and the possibility of their formation in different tissues.
- Several general equations can be used to determine the reaction order of alanine aminotransferase (AAP) isomers I and II.
- Thermodynamic studies show that the driving force of the binding reactions depends on the entropy change function (ΔH), indicating the importance of hydrophobic effects in the formation of the resulting complexes.
- The isolated AAP enzyme isoforms and their associated complexes can be distinguished using spectroscopic studies.

Recommendations:

1. Expand the investigation of alanine aminotransferase (AAP) activity and its isoforms in the urine of patients with other urological cancers, such as bladder cancer, ureteral cancer, and others.
2. Conduct extensive studies to determine the source of isoforms I and II in the urine of patients with renal impairment.
3. Perform kinetic and thermodynamic studies of isoforms I and II in various urological cancers.

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