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THERMOCHEMISTRY OF THE L-ASPARAGINE HYDROLYSIS REACTION CATALYZED BY L-ASPARAGINASE

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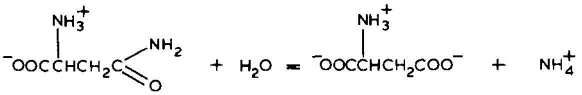
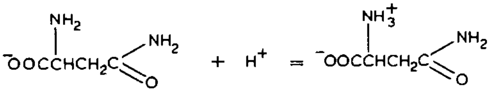
ABSTRACT

The enthalpy of the asparagine hydrolysis reaction



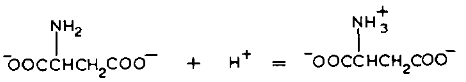
$$ {}^{-}OOC\overset{\overset{NH_3^+}{|}}{C}HCH_2C\begin{smallmatrix}NH_2\\ O\end{smallmatrix} + H_2O = {}^{-}OOC\overset{\overset{NH_3^+}{|}}{C}HCH_2COO^- + NH_4^+ $$

is found to be $\Delta H_1 = -24.56 \pm 0.35$ kJ mol^{-1} (0.05 M phosphate buffer, pH 6.86, 298.15 K); besides, the enthalpies of ionization of asparagine and aspartic acid are also determined



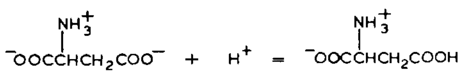
$$ {}^{-}OOC\overset{\overset{NH_2}{|}}{C}HCH_2C\begin{smallmatrix}NH_2\\ O\end{smallmatrix} + H^+ = {}^{-}OOC\overset{\overset{NH_3^+}{|}}{C}HCH_2C\begin{smallmatrix}NH_2\\ O\end{smallmatrix} $$

$\Delta H_2 = -39.7 \pm 0.3$ kJ mol^{-1}



$$ {}^{-}OOC\overset{\overset{NH_2}{|}}{C}HCH_2COO^- + H^+ = {}^{-}OOC\overset{\overset{NH_3^+}{|}}{C}HCH_2COO^- $$

$\Delta H_3 = -38.6 \pm 0.4$ kJ mol^{-1}



$$ {}^{-}OOC\overset{\overset{NH_3^+}{|}}{C}HCH_2COO^- + H^+ = {}^{-}OOC\overset{\overset{NH_3^+}{|}}{C}HCH_2COOH $$

$\Delta H_4 = -3.83 \pm 0.10$ kJ mol^{-1}

Using values of the enthalpies ΔH_1 to ΔH_4, the plot of enthalpy variation of the asparagine hydrolysis reaction versus pH (3–10) is plotted.

INTRODUCTION

L-Asparagine, one of the main amino acids, participates in diverse reactions that occur in living organisms.

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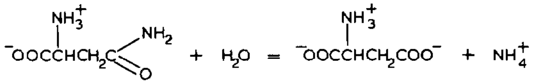
This work deals with the microcalorimetric assessment of the enthalpy of the L-asparagine enzymic hydrolysis reaction at pH 3–10



$$^{-}OOC\overset{NH_3^+}{\overset{|}{C}}HCH_2C\genfrac{}{}{0pt}{}{\diagup NH_2}{\diagdown O} + H_2O = {}^{-}OOC\overset{NH_3^+}{\overset{|}{C}}HCH_2COO^- + NH_4^+ \qquad (1)$$

Catalyzed by L-asparaginase enzyme, this reaction is extremely vital for the active life of leucosis asparagine-dependent cells. The L-asparaginase enzyme is available in a very pure form, since in the U.S.S.R. it has been administered as an antitumourous medicinal.

Previous thermochemical investigations of reaction (1), carried out in ref. 1 with a unique microcalorimetric apparatus [2–4], had certain methodic shortcomings: reaction (1) proceeded at pH 8.5; under such conditions both asparagine and aspartic acid were present in the solution in two different ionic forms. For this reason, when calculating ΔH_1, the authors of ref. 1 had to introduce special corrections without, however, specifying the errors involved. The asparaginase enzyme compounds, used in ref. 1, were not investigated in full, so that side reactions could not be excluded.

In this work, the ΔH_1 value has been determined with the aid of a flow microcalorimeter (LKB-2107-121) and the methods used differed in principle from those of ref. 1; the ΔH_1 vs. ambient pH relationship has also been investigated. Besides ΔH_1 we have assessed the ionization enthalpies of substrate and products of reaction (1).

EXPERIMENTAL

Materials

A sample of L-asparaginase from the Riga Medicinal Factory had the following characteristics: specific activity, 290 international units (IU); main admixtures: aminoacetic acid, 56 and water, 4%. With the exception of aspartic acid, no organic substances have been traced in the products of reaction (1). The compounds of L-asparagine ($C_4H_8O_3N_2 \cdot H_2O$; MW 150.13) and aspartic acid ($C_4H_7O_4N$; MW 133.12) (Reanal, Hungary) have proved to be chromatographically pure. We carried out an elementary analysis from which it follows that the contents of nitrogen, carbon and hydrogen in the samples used agree better than 0.5% with the theoretical values and the angle of optical rotation with that of ref. 5.

Methods

The enthalpy of the L-asparagine hydrolysis reaction has been determined in a flow microcalorimeter (LKB-2107-121) according to the method described earlier [6]. According to this method, an LKB-10200 pump was used to introduce a 0.05 M phosphate buffer solution containing L-asparaginase enzyme at 5.5 g h^{-1} into a reaction cell for mixing, while a similar pump was used to introduce a buffer solution containing substrate. The concentrations of enzyme and substrate were varied during the experiments.

A correction for the dilution of the original substrate solution was automatically introduced along with the method used [6]. As for the correction for diluting the original enzyme solution, it was less than the thermal noise of the instrument.

A standard phosphate buffer solution (0.05 M, pH 6.86) was used in all experiments. At an accepted pH value, all the components of reaction (1) are present in the solution predominantly (98–99%) in one ionic form.

The ionization enthalpy of asparagine and aspartic acid was determined with the aid of a flow microcalorimeter (LKB-2107-121) following ref. 7. The pH value of the original solution of asparagine and aspartic acid intended to carry out the microcalorimetric studies was chosen so that 80–90% of the reagent was in the dissociated state. When calculating the ionization enthalpy a correction was introduced on the assumption that not all the hydrochloric acid reacted with asparagine and aspartic acid: part of it was used to change the pH of the final solution with respect to the original value.

RESULTS AND DISCUSSION

The results of determining the enthalpy of the L-asparagine hydrolysis reaction in the presence of L-asparaginase are presented in Table 1; the results of the electrical calibration of the flow microcalorimeter are given in arbitrary units (obtained through differential recording in an LKB-2066 recorder) and in μW. Also cited is enzyme activity (IU), original concentration of substrate (asparagine; μmol g^{-1}) and flow rate of substrate (g h^{-1} and mol s^{-1} 10^{-9}). In the last column of Table 1, the enthalpy values, ΔH_1, for each experiment (kJ mol^{-1}) are given. As is clear from Table 1, ΔH_1 does not alter the concentration of enzyme (within 440–110 IU) and substrate (within 2–12 mol g^{-1}). The ΔH_1 value calculated from the data of Table 1 is -24.56 ± 0.35 kJ mol^{-1}. This value agrees within error tolerance with the results of ref. 1 (-23.9 ± 0.5 kJ mol^{-1}). Hence, two different microcalorimetric method give values of ΔH_1 in good agreement with each other.

In order to calculate variations of ΔH_1 depending on pH, one should know the values of the ionization enthalpies of the components of reaction (1) (these values were determined by us experimentally; see Tables 2–4). The tables contain also critically selected (on the basis of data [8]) values of dissociation constants (pK) of substrate and product of reaction (1): 8.75 for asparagine; 3.74 and 9.71 for aspartic acid.

The results of calculating the ionization enthalpy of asparagine, according to the foregoing dissociation constant, are given in Table 2.

TABLE 1

Enthalpies obtained for the reaction (ΔH_1) of hydrolysis of asparagine in the presence of L-asparaginase (0.05 M phosphate buffer solution, pH 6.86, 298.15 K)

Q_c		Q_{exp}		Enzyme conc. (IU g^{-1})	Asparagine conc. ($\mu\text{M g}^{-1}$)	Asparagine flow rate		ΔH_1 [a] (kJ mol^{-1})
(arb. units)	(μW)	(arb. units)	(μW)			(g h^{-1})	(mol s^{-1} $\times 10^{-9}$)	
160.6	451.4	152.5	428.6	440	11.61	5.29	17.06	−25.12
160.5	451.4	153.7	432.3	154	11.61	5.29	17.06	−25.34
159.5	451.4	150.6	426.2	154	11.61	5.29	17.06	−24.98
81.0	451.4	71.5	398.5	340	11.09	5.33	16.42	−24.27
81.0	451.4	71.0	395.7	290	11.09	5.33	16.42	−24.10
81.0	451.4	72.0	401.2	300	11.09	5.33	16.42	−24.43
81.0	451.4	76.0	423.5	300	11.71	5.29	17.21	−24.61
81.0	451.4	75.5	420.7	110	11.71	5.29	17.21	−24.45
81.0	451.4	46.0	256.4	300	7.112	5.33	10.53	−24.35
81.0	451.4	46.8	260.8	110	7.112	5.33	10.53	−24.77
81.0	451.4	24.5	136.5	300	3.679	5.33	5.447	−25.06
81.0	451.4	23.5	131.0	110	3.679	5.33	5.447	−24.05
60.3	112.9	67.0	125.4	110	3.357	5.55	5.175	−24.23
60.3	112.9	54.5	102.0	110	2.673	5.55	4.121	−24.75
60.3	112.9	35.5	66.5	110	1.769	5.55	2.727	−24.39
60.3	112.9	35.0	65.5	110	1.769	5.55	2.727	−24.02
							Average	−24.56 ± 0.35

[a] $\Delta H_1 = -(Q_{exp} m^{-1})$, where Q_{exp} = heat released during the experiment (μW) and m = quantity of substrate used ($\text{mol s}^{-1} \times 10^{-9}$).

TABLE 2

Enthalpies obtained for the reaction (ΔH_2) of asparagine ionization (298.15 K, pK 8.75)

Q_c		Q_{exp}		Asparagine conc. ($\mu\text{M g}^{-1}$)	HCl conc. ($\mu\text{M g}^{-1}$)	HCl flow rate		ΔH_2 [a] (kJ mol^{-1})
(arb. units)	(μW)	(arb. units)	(μW)			(g h^{-1})	(mol s^{-1} $\times 10^{-9}$)	
56.0	313.5	31.7	177.5	57.1	2.93	5.44	4.43	−40.07
56.0	313.5	89.7	502.2	57.1	8.36	5.44	12.63	−39.76
56.0	313.5	31.5	176.3	57.1	2.93	5.44	4.43	−39.80
56.0	313.5	56.6	316.9	57.1	5.26	5.44	7.95	−39.86
56.0	313.5	90.2	505.0	28.3	8.36	5.44	12.63	−39.98
56.0	313.5	31.0	173.5	28.3	2.93	5.44	4.43	−39.16
56.0	313.5	56.2	314.6	28.3	5.26	5.44	7.95	−39.57
							Average	−39.7 ± 0.3

[a] See footnote to Table 1.

TABLE 3

Enthalpies obtained for the reaction (ΔH_3) of aspartic acid ionization (298.15 K, pK 9.71)

Q_c		Q_{exp}		Aspartic acid conc. (μM g^{-1})	HCl conc. (μM g^{-1})	HCl flow rate		ΔH_3 [a] (kJ mol^{-1})
(arb. units)	(μW)	(arb. units)	(μW)			(g h^{-1})	(mol s^{-1} $\times 10^{-9}$)	
56.0	313.5	31.0	173.5	30.0	2.93	5.46	4.44	−39.08
56.0	313.5	48.0	268.7	30.0	4.59	5.46	6.96	−38.61
56.0	313.5	84.5	473.0	30.0	8.13	5.46	12.33	−38.36
56.0	313.5	21.0	117.6	60.0	2.00	5.46	3.03	−38.81
56.0	313.5	59.0	330.3	60.0	5.84	5.46	8.86	−37.28
56.0	313.5	77.5	433.9	60.0	7.23	5.46	10.97	−39.55
							Average	−38.6 ± 0.4

[a] See footnote to Table 1.

Table 2 sums up the results of electrical calibration of the microcalorimeter (arbitrary units and μW), the heat liberated during the experiment (arbitrary units and μW), the original concentration of asparagine solution (μmol g^{-1}), the concentration of HCl (μmol g^{-1}), and the flow rate of HCl (g h^{-1} and mol s^{-1} $\times 10^{-9}$). The last column of Table 2 gives the values of asparagine ionization enthalpy for each experiment (kJ mol^{-1}).

Tables 3 and 4 sum up the results of determining the aspartic acid ionization enthalpy from eqns. (3) and (4).

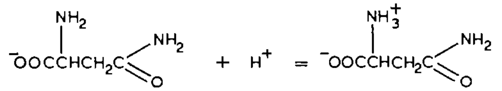
In our experiments we have obtained the following values for the ionization enthalpies of asparagine and aspartic acid

TABLE 4

Enthalpies obtained for the reaction (ΔH_4) of aspartic acid ionization (298.15 K, pK 3.74)

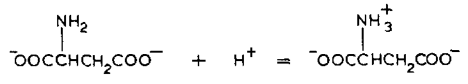
Q_c		Q_{exp}		Aspartic acid conc. (μM g^{-1})	HCl conc. (μM g^{-1})	HCl flow rate		ΔH_4 [a] (kJ mol^{-1})
(arb. units)	(μW)	(arb. units)	(μW)			(g h^{-1})	(mol s^{-1} $\times 10^{-9}$)	
59.5	32.1	32.0	17.3	60.0	3.10	5.46	4.70	−3.68
59.5	32.1	76.5	41.3	30.0	6.86	5.46	10.40	−3.97
59.5	32.1	52.5	28.3	30.0	5.08	5.46	7.70	−3.68
27.0	50.2	29.5	54.8	30.0	9.22	5.46	13.98	−3.92
59.0	32.1	31.5	17.1	60.0	2.93	5.46	4.44	−3.85
59.0	32.1	49.5	26.9	60.0	4.59	5.46	6.96	−3.86
59.0	32.1	69.5	37.8	60.0	6.51	5.46	9.87	−3.83
							Average	−3.83 ± 0.10

[a] See footnote to Table 1.



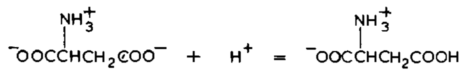
(2)

$\Delta H_2 = -39.7 \pm 0.3$ kJ mol^{-1}



$$^{-}OOCCH(NH_2)CH_2COO^{-} + H^{+} = ^{-}OOCCH(NH_3^{+})CH_2COO^{-} \quad (3)$$

$\Delta H_3 = -38.6 \pm 0.4$ kJ mol^{-1}



$$^{-}OOCCH(NH_3^{+})CH_2COO^{-} + H^{+} = ^{-}OOCCH(NH_3^{+})CH_2COOH \quad (4)$$

$\Delta H_4 = -3.83 \pm 0.10$ kJ mol^{-1}

For the ionization enthalpies (ΔH_i) considered by us in reactions (2), (3) and (4), the following values are given in the literature: $\Delta H_2 = -40.6$ [8], $\Delta H_3 = -37.8$ [9], $\Delta H_4 = -4.6$ [9], $\Delta H_4 = -6.4 \pm 0.15$ kJ mol^{-1} [1]. The foregoing values of ΔH_i coincide with our data within 1 kJ mol^{-1}, except the results of ref. 1. A concise description of the experimental data in ref. 1 makes it impossible to analyse the experimental data underlying that work in detail. Subsequently, we have used for our calculations the value $\Delta H_4 = -3.83 \pm 0.10$ kJ mol^{-1}, obtained in the present work.

On the ground of experimentally obtained values of ΔH_1, ΔH_2, ΔH_3 and

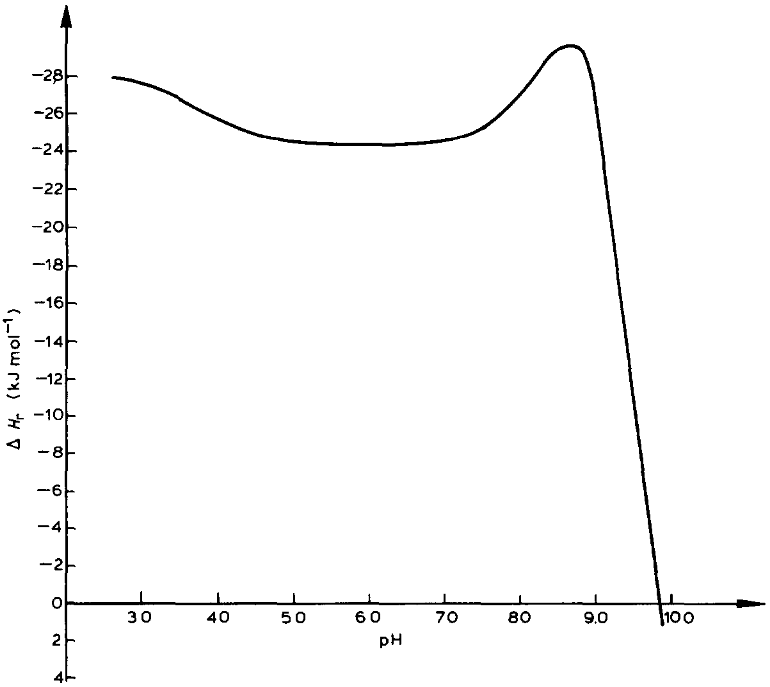


Fig. 1. The enthalpy variation of asparagine hydrolysis enzymic reaction versus pH.

ΔH_4 and also using literature data on the dissociation constants of reaction (1) components, we have calculated (assuming $c = a$, where c = concentration, a = activity) the profile of the enthalpy variation for asparagine hydrolysis reaction versus pH of the medium (see Fig. 1). The relationship of the enthalpy variation versus pH of medium, shown in Fig. 1, has its minimum in the range of pH 5–7. In this range of pH the reaction can be presented through eqn. (1). At pH < 5, association of aspartic acid anion (charge -1) with H^+ occurs. Since this process is exothermic ($\Delta H_4 = -3.83 \pm 0.10$ kJ mol^{-1}), it follows that the summary variation of the enthalpy of the asparagine enzymic hydrolysis reaction becomes more exothermal. At pH > 7, asparagine dissociates. With pH varying from 7 to 8.6 this process will induce a greater liberation of heat (see Fig. 1). At pH > 8.6, an essential contribution to the summary value of the reaction enthalpy will be made by endothermal processes that occur during the dissociation of ammonium ion and aspartic acid. As a result, with pH rising from 8.6 to 10, the summary value of the enthalpy will become less exothermal and at pH ~ 9.8 the summary effect will become zero; at higher pH (9.9–10.0) the summary value of the reaction under consideration will become endothermal.

CONCLUSIONS

The profile of the enthalpy variation for the asparagine hydrolysis reaction versus pH (3–10) was calculated from the values of enthalpies ΔH_1–ΔH_4 (these values were determined by us experimentally); $\Delta H_1 = -24.56 \pm 0.35$, $\Delta H_2 = -39.7 \pm 0.3$, $\Delta H_3 = -38.6 \pm 0.4$, $\Delta H_4 = -3.83 \pm 0.10$ kJ mol^{-1}. The results of these calculations were important for the development of a microcalorimetric method of asparaginase activity determination at different pH. The sensitivity of the microcalorimetric method was proportional to the value of the reaction enthalpy.

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