Thermochimica Acta, 81 (1984) 167–174
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

THERMOCHEMISTRY OF THE *N*-ACETYLPHENYLALANINE METHYLESTER HYDROLYSIS REACTION CATALYZED BY α-CHYMOTRYPSIN

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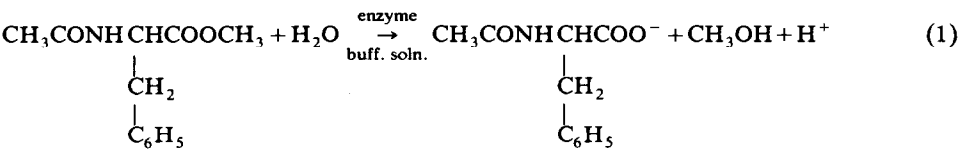
(Received 13 June 1984)

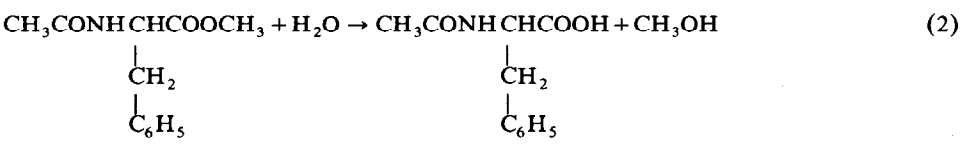
ABSTRACT

The enthalpy of the reaction



(1)

is found to be $\Delta H_1 = -4.81 \pm 0.10$ kJ mol^{-1} (0.05 M phosphate buffer, pH 7.0, 298.15 K). Using values of the enthalpies of ionization of the buffer solution ($\Delta H^i_{buffer} = -3.98 \pm 0.20$ kJ mol^{-1}) and *N*-acetylphenylalanine ($\Delta H^i_{acphen} = -2.46 \pm 0.09$ kJ mol^{-1}), found experimentally, the enthalpy of the following hydrolysis reaction has been calculated



(2)

$\Delta H_2 = \Delta H_1 - \Delta H^i_{buff} - \Delta H^i_{acphen} = +1.63 \pm 0.25$ kJ mol^{-1}, as applied to the reaction of undissociated compounds in aqueous solution. The enthalpy of reaction (2) is compared to the enthalpies of other hydrolysis reactions in compounds with ester bonds.

INTRODUCTION

The hydrolysis reaction under investigation belongs, according to the IUB international nomenclature, to the third class of enzymatic reactions [1].

Earlier, a principle for developing a system of key thermodynamic data for enzymology was proposed [2]. According to this principle the system of data and nomenclature of enzymes will correspond to the IUB classification, all enzymes being classified into six large groups: (1) oxidoreductases; (2) transferases; (3) hydrolases; (4) liases; (5) isomerases; (6) ligases. Earlier, the key thermodynamic values for the first class of reactions catalyzed by oxidoreductases were determined [3].

0040-6031/84/$03.00

Calculations for determining thermodynamic parameters for the third class of reactions catalyzed by hydrolases can be facilitated by using mean values $\Delta\overline{H}_r$ and $\Delta\overline{G}_r$ for the hydrolysis of compounds with the same type of hydrolyte bond, e.g., ester bond [2].

In this paper, the enthalpy of the hydrolysis reaction of *N*-acetylphenylalanine methyl ester catalyzed by α-chymotrypsin in a phosphate buffer solution (the hydrolysis of the ester bond) is determined. The results of work are compared to literature data pertinent to the enthalpy of the hydrolysis reaction with the ester bond as it takes place in other compounds.

EXPERIMENTAL

The enthalpy of the hydrolysis reaction was determined using an LKB-2107-batch microcalorimeter (LKB-2107-111) and a flow-type microcalorimeter (LKB-2107-121).

The substrate, *N*-acetylphenylalanine methyl ester, was synthesized and rectified by previously described methods [4]. Two chromatographically pure specimens of substrate were taken for thermochemical analysis: t_{melt} = 90–91°C, $[\alpha]^{25}$ + 19° (2% methanol).

The hydrolysis reaction was conducted in 0.05 M phosphate buffer solution (pH 7, 298.15 K). The original concentration of substrate was 2–6 μmol g^{-1}, and the enzyme (α-chymotrypsin) was within 0.06–2.2 mg g^{-1}. Under the foregoing conditions the hydrolysis reaction proceeds, both in character and quantitatively [5], as described by eqn. (1)

$$\begin{array}{l} CH_3CONHCHCOOCH_3 + H_2O \\ \qquad\quad | \\ \qquad\quad CH_2 \\ \qquad\quad | \\ \qquad\quad C_6H_5 \end{array} \xrightarrow[\text{buff. soln.}]{\text{enzyme}} \begin{array}{l} CH_3CONHCHCOO^- + CH_3OH + H^+ \\ \qquad\quad | \\ \qquad\quad CH_2 \\ \qquad\quad | \\ \qquad\quad C_6H_5 \end{array} \qquad (1)$$

When carrying out the experiments in a batch microcalorimeter, one part of the reaction cell was charged with 2 g of buffer solution containing 0.1 mg g^{-1} α-chymotrypsin, while the other part of the reaction cell was charged with 4 g of the same buffer solution plus 2–6 μmol g^{-1} *N*-acetylphenylalanine methyl ester. After having reached thermal equilibrium, the solutions were mixed and the hydrolysis reaction occurred (1). The measured thermal effect was corrected for the frictional heat of the solution of the reaction cell.

The experiments on a flow-type microcalorimeter were conducted as

follows. A buffer solution, containing 4–5 μmol g^{-1} *N*-acetylphenylalanine methyl ester, was fed into the flow cell for mixing by the LKB-10200 pump at a flow rate of 5.5 g h^{-1}. Another pump, at the same flow rate, feeds a similar buffer solution containing 0.06–2.2 mg g^{-1} α-chymotrypsin. The higher the concentration of enzyme in the buffer solution after mixing, the greater the rate at which the reaction under consideration runs and, consequently, the larger the percentage of substrate that manages to react before the solution leaves the cell. After the concentration had reached a certain level, any further rise in concentration had no heat effect; in this case it was concluded that the hydrolysis of substrate had run completely.

The enthalpy of the ionization of *N*-acetylphenylalanine was assessed with the assistance of a flow-type microcalorimeter, in which a 0.03–0.06 M solution of *N*-acetylphenylalanine at pH 4.9 was mixed with a 0.003–0.008 N solution of HCl. Methods for assessing the enthalpy of ionization of the phosphate buffer solution were described earlier [6].

RESULTS AND DISCUSSION

Table 1 contains the results of experiments made to assess the enthalpy of the enzymatic hydrolysis reaction of *N*-acetylphenylalanine methyl ester in a batch-type microcalorimeter.

The table lists the results of the electric calibration, Q_c, of the microcalorimeter in arbitrary units (integral records on the LKB-2066 potentiometric recorder) and in J; heat released during the experiments, Q_{exp}, was also recorded in arbitrary units and in J; a correction for the frictional heat of the solution inside the reaction cell, given in arbitrary units, was determined separately. The table also specifies (for each experiment) the original concentration of substrate (μmol g^{-1}), the mass of substrate solution in the reaction cell (g) and the amount of substrate that has managed to react (μmol); the last column of Table 1 gives the enthalpy values (kJ mol^{-1}) for each experiment of the reaction under analysis.

The enthalpy of the hydrolysis reaction of *N*-acetylphenylalanine methyl ester was found with the assistance of a batch-type microcalorimeter to be -4.80 ± 0.10 kJ mol^{-1} (Table 1).

Table 2 contains the results of the electric calibration of a flow-type microcalorimeter, Q_c, which are given in arbitrary units (integral records on the LKB-2066 recorder) and in μW; heat release values, Q_{exp}, are given in arbitrary units, in μW and in J h^{-1}. Table 2 also specifies (for each experiment) the original concentration of enzyme (mg g^{-1}); the flow rate of enzymatic solution through the reaction microcalorimetric cell (g h^{-1}); the original concentration of substrate (μmol g^{-1}); the flow rate of the substrate solution (g h^{-1}); and the number of moles of substrate running through the cell per hour. The last column of the table gives the enthalpies (kJ mol^{-1}) for each experiment of the reaction under analysis.

TABLE 1

Enthalpies obtained for the reaction (ΔH_r) of hydrolysis of *N*-acetylphenylalanine methyl ester in an LKB-2107 batch microcalorimeter (298.15 K; pH 7.0; 0.05 M phosphate buffer solution)

No.	Q_c		Q_{exp}		Correction for friction (arbitrary units)	Substrate			ΔH_r [a] (kJ mol^{-1})
	Arbitrary units	J	Arbitrary units	(J)		Original concentration (μM g^{-1})	Mass of solution used in experiment (g)	Amount of substrate used in experiment, m (μM)	
1	271.9	0.1350	196.9	0.0946	6.4	4.952	3.998	19.80	−4.78
2	219.2	0.1094	150.1	0.0717	6.5	4.952	3.007	14.89	−4.82
3	120.2	0.0603	115.2	0.0547	6.1	2.949	3.968	11.70	−4.68
4	217.6	0.1094	120.2	0.0575	5.9	2.949	3.974	11.72	−4.91
5	274.5	0.1350	209.2	0.0996	6.6	5.437	3.954	21.50	−4.63
6	109.2	0.0603	168.5	0.0887	7.8	4.632	3.929	18.20	−4.87
7	188.5	0.0940	185.9	0.0905	4.5	4.632	3.965	18.37	−4.93

Average value -4.80 ± 0.10

[a] $\Delta H_r = -(10^3\ Q_c\ m^{-1})$ kJ mol^{-1}, where Q_c = heat released in experiment (J) and m = quantity of substrate used (μM).

TABLE 2

Enthalpies obtained for the reaction of enzymatic hydrolysis of *N*-acetylphenylalanine methyl ester in a flow-type microcalorimeter LKB-2107 (298 K; pH 7.0; 0.5 M phosphate buffer solution)

No.	Q_c		Q_{exp}			Enzyme		Substrate			$-\Delta H_r$ [a] (kJ mol^{-1})
	Arbitrary units	μW	Arbitrary units	μW	J h^{-1}	Concentration (mg ml^{-1})	Flow rate (g h^{-1})	Concentration (mM)	Flow rate (g h^{-1})	μM h^{-1}; *m*)	
1	79.0	24.6	40.1	12.5	0.0448	0.055	5.64	4.30	5.10	21.9_5	2.04
2	112.6	32.1	63.7	18.1_5	0.0654_5	0.059	5.10	4.63	5.63	26.1	2.51
3	79.0	24.6	66.2	20.6	0.0740	0.074	5.63	4.30	5.10	21.9_5	3.37
4	93.5	24.6	104.8	27.6	0.0995	0.15	5.65	4.52	5.10	23.0_5	4.32
5	79.0	24.6	87.5	27.2_5	0.0981	0.24	5.65	4.30	5.08	21.8_5	4.49
6	113.0	32.1	113.5	32.2	0.1161	0.34	5.09	4.63	5.63	26.1	4.45
7	79.0	24.6	92.9	28.9_5	0.1043	0.43	5.63	4.30	5.10	21.9_5	4.75
8	121.0	32.1	123.5	32.7_5	0.1179	0.55	5.66	4.52	5.10	23.0_5	5.11
9	84.3	24.6	116.3	34.0	0.1223	0.55	5.09	4.63	5.63	26.1	4.69
10	57.3	36.2_5	49.6	31.4	0.1130	0.86	5.65	4.53	5.21	23.6	4.79
11	93.0	24.6	121.0	32.0	0.1152	1.03	5.66	4.52	5.10	23.0_5	5.00
12	79.0	24.6	95.0	29.0	0.1065	1.20	5.66	4.30	5.09	21.9	4.86
13	57.3	36.2_5	50.4	31.9	0.1148	1.30	5.65	4.53	5.21	23.6	4.86
14	57.3	36.2_5	48.4	30.6	0.1102	1.46	5.65	4.53	5.21	23.6	4.67
15	79.0	24.6	92.3	28.7_5	0.1035	2.24	5.65	4.30	5.08	21.8_5	4.74

Average 4.83 ± 0.13

[a] See footnote to Table 1.

For better clarity the data in Table 2 are plotted graphically in Fig. 1 demonstrating the relationship between the heat release in the microcalorimetric cell (correlated with substrate mol) and the original concentration of enzyme.

Heat release was found to increase with increasing enzyme concentration (0.06–0.35 mg g^{-1}). An enzyme concentration of < 0.4 mg g^{-1} is not sufficient (Fig. 1) for a complete hydrolysis of the substrate within the time the solution remains inside the cell (ca 2.5 min). At an enzyme concentration of 0.4–2.2 mg g^{-1} the relationship of heat release versus enzyme concentration is absent. In this case the reaction runs to the end. To calculate the enthalpy of reaction (1) use was made of the results of experiments in which enzyme concentration exceeded 0.4 mg g^{-1}.

The parameters of the hydrolysis reaction of *N*-acetylphenylalanine methyl ester, determined with the assistance of a batch microcalorimeter (-4.80 ± 0.10 kJ mol^{-1}) and a flow-type microcalorimeter (-4.83 ± 0.13 kJ mol^{-1}), correlate well with each other. This is why the average value $\Delta H_p = -4.81 \pm 0.10$ kJ mol^{-1} has been taken for the reaction (1) running in buffer solution (pH 7, 298.15 K). This value is suitable for use in biochemistry. For calculating the standard value of the enthalpy of the hydrolysis reaction it was also necessary to assess the ionization enthalpy of *N*-acetylphenylalanine

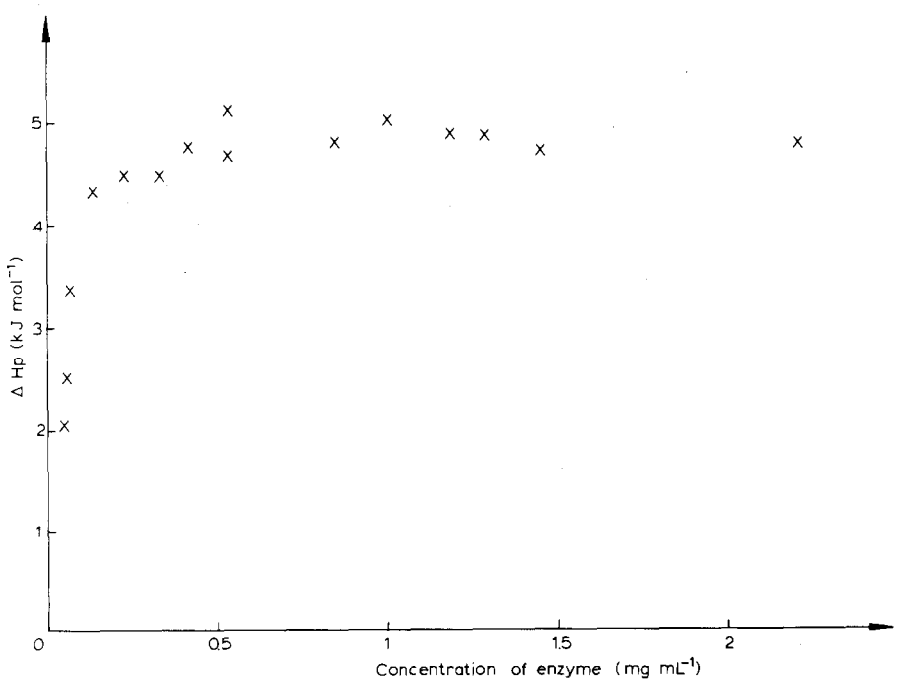


Fig. 1. Heat release in microcalorimetric cell (related to moles of substrate) versus original concentration of enzyme.

TABLE 3

Enthalpies of the hydrolysis reaction in compounds with ester bonds

Compound	ΔH_p (kJ mol^{-1})	Ref.
$CH_3COOCH_2CH_2NHCOCH_3$	1.97 ± 0.30	7
$CH_3COOCH_2CH_3$	1.80 ± 0.25	7
$CH_3CONH\underset{\substack{\vert\\ CH_2\\ \vert\\ C_6H_5}}{CH}COOCH_3$	1.63 ± 0.25	This work

and buffer solution reactions. The enthalpy value of buffer solution ionization was found to equal $\Delta H^{i}_{buff} = -3.98 \pm 0.20$ kJ mol^{-1}. The enthalpy of ionization of *N*-acetylphenylalanine was determined experimentally to be $\Delta H^{i}_{acphen} = -2.46 \pm 0.09$ kJ mol^{-1}.

Thus, the enthalpy of the hydrolysis reaction of *N*-acetylphenylalanine methyl ester

$$CH_3CONH\underset{\substack{|\\ CH_2\\ |\\ C_6H_5}}{CH}COOCH_3 + H_2O = CH_3CONH\underset{\substack{|\\ CH_2\\ |\\ C_6H_5}}{CH}COOH + CH_3OH \qquad (2)$$

equals $\Delta H_2 = \Delta H_1 - \Delta H^{i}_{buff} - \Delta H^{i}_{acphen} = +1.63 \pm 0.25$ kJ mol^{-1} for the reaction of undissociated molecules in an aqueous solution.

Table 3 contains the enthalpies of the hydrolysis reaction in compounds with ester bonds. The values clearly correlate well, within the error admitted. The average value of the enthalpy of ester bond hydrolysis is, according to Table 3, $\Delta H_p = +1.80 \pm 0.25$ kJ mol^{-1}.

CONCLUSIONS

The enthalpy of the hydrolysis reaction of *N*-acetylphenylalanine methyl ester is found to be $\Delta H_2 = 1.63 \pm 0.25$ kJ mol^{-1} as applied to the reaction of undissociated molecules in aqueous solution.

The enthalpy values obtained for certain reactions are compared with the literature data for the enthalpies of hydrolysis reactions in compounds with an ester bond. The average value of the enthalpy of ester bond hydrolysis was found to be 1.80 ± 0.25 kJ mol^{-1}.

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