Thermochimica Acta, 112 (1987) 151–160
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

THERMOCHEMISTRY OF HYDROLYTIC ENZYMATIC REACTIONS

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(Received 9 May 1986)

ABSTRACT

On the basis of literature data and from our own experiments, the average enthalpies ($\Delta \overline{H}_r$) for different types of hydrolytic enzymatic reactions have been found: for peptide bond hydrolysis $\Delta \overline{H}_r = -7.6 \pm 2.0$ kJ mol^{-1}; for amide bond hydrolysis $\Delta \overline{H}_r = -24.7 \pm 2.0$ kJ mol^{-1}; for ester bond hydrolysis $\Delta \overline{H}_r = 1.8$ kJ mol^{-1}. Furthermore, $\Delta \overline{H}_r$ values have been calculated for various types of hydrolysis reaction in orthophosphate esters and their derivatives; $\Delta \overline{H}_r$ values for other types of hydrolytic enzymatic reactions are also cited from the literature.

INTRODUCTION

Hydrolytic enzymatic reactions make up an extensive class of biochemical reactions which proceed without cofactors [1,2]. The absence of cofactors in this case rules out a principle that could otherwise be employed for calculating the enthalpy of reactions which was used by us earlier in dealing with enzymatic reactions catalyzed by oxidoreductases [3]. The enthalpies of hydrolytic enzymatic reactions can be calculated much more easily if average enthalpy values ($\Delta \overline{H}_r$) of hydrolytic reactions are applied to compounds in which a similar type of bond is hydrolyzed, i.e., esters, peptides, etc. [4].

This work gives a method for determining average enthalpies ($\Delta \overline{H}_r$) for the most important types of hydrolytic reactions: hydrolysis of peptides, amides, orthophosphates, esters, etc. Thus, $\Delta \overline{H}_r$ values, cited in this paper, cover the hydrolysis processes in bonds contained in the basic components of living matter, i.e., proteins, nucleic acids, fats, carbohydrates.

THERMOCHEMISTRY OF HYDROLYTIC REACTIONS IN PEPTIDES AND AMIDE BONDS

Sturtevant and co-workers [5–10] were the first to obtain credible enthalpies of hydrolysis reactions for more than ten peptides and amides, thus making a substantial contribution to the thermochemistry of hydrolytic

0040-6031/87/$03.50

TABLE 1

Enthalpies of hydrolytic reactions of peptide-bonded compounds (ΔH_r, 298.15 K)

Compound	Bond hydrolyzed	ΔH_r (kJ mol^{-1})	Ref.
Poly-L-lysine	Lysine–lysine	−5.20	7
L-Tyrosylglycinamide	Tyrosine–glycine	−5.44 ± 0.65	9
Benzoyl-L-tyrosylglycine	Tyrosine–glycine	−5.56 ± 0.40	9
Benzoyl-L-tyrosyl-glycineamide	Tyrosine–glycine	−6.49 ± 0.65	6
Benzoyl-L-tyrosine	Benzoic acid–tyrosine	−8.28 ± 0.40	9
Phenylacetyl-L-phenylglycine	Phenylacetic acid–phenylglycine	−8.58 ± 0.20	This work
Carbobenzoxyglycyl-L-leicine	Glycine–leucine	−8.83 ± 0.20	8
Benzoyl-L-tyrosyl-glycineamide	Benzoic acid–tyrosine	−9.33 ± 0.90	9
Carbobenzoxyglycyl-L-phenylalanine	Glycine–phenylalanine	−10.67 ± 0.20	5

reactions in peptide and amide bonds. The results of their studies on the determination of enthalpies of hydrolytic reactions for various peptides are presented in Table 1.

The Table also gives the enthalpy of hydrolytic reaction for peptide bonds in phenylacetyl–phenylglycine (APPG)–$C_6H_5CH_2CONHCH(C_6H_5)COONa$, which was found experimentally in this work. The ΔH_r (APPG) value was found from the data in Table 2.

Table 2 sums up the results of electric calibration in provisional units (differential record on an LKB-2066 recorder) and in μW, and heat evolution in the course of experiment (in provisional units and in μW). Table 2 also gives: concentration of the enzyme penicillinammidase in the initial solution in (international units, IU), initial concentration of the substrate (mM), and substrate flow rate (g h^{-1} and mol s^{-1} 10^{-9}). The last column in Table 2 (for each experiment) specifies enthalpies for the APPG hydrolytic reaction.

Microcalorimetric experimental methods, in which a flow microcalorimeter is involved, have been described previously [11]; DL-phenylacetyl–phenylglycine specimens were used for the experiments (Table 2). The specimens were optically inactive and comprised 50% of D-isometer and 50% of L-isomer. Fig. 1 represents diagrammatically the results of experiments, in which a mixture of DL-isomers (experiments 1 and 2, Table 2) and D-isomer (the specimen contained 98–99% of D-isomer and 1–2% of L-isomer) were used. It is evident that the enzyme used (penicillinamidase) catalyzes (under experimental conditions) the hydrolysis of the L-isomer only. This is why Table 2 specifies only the concentration of the L-isomer in solution rather than the total concentration of substrate.

TABLE 2

Enthalpy of hydrolytic reaction in acetophenyl–phenylglucine (flow microcalorimeter LKB-2107; 298.15 K; 0.05 M phosphate buffer solution; pH 6.86)

Exp. no.	Q_{calib}		Q_{exp}		Concentration		Substrate flowrate (V)		ΔH_p [a] (kJ mol^{-1})
	Provisional unit	μW	Provisional unit	μW	Enzyme (IU)	Substrate (mM)	g h^{-1}	mol^{-1} s^{-1} 10^{-9}	
1	65.5	36.2	40.1	22.2	1000	1.74	5.50	2.66	−8.35
2	65.5	36.2	70.8	39.1	1000	3.04	5.50	4.64	−8.43
3	65.5	36.2	29.0	16.0	1000	1.18	5.50	1.80	−8.89
4	65.5	36.2	55.0	30.4	1000	2.32	5.50	3.54	−8.59
5	51.0	28.2	74.0	40.9	1300	3.02	5.53	4.64	−8.81
6	51.0	28.2	42.3	23.4	1300	1.80	5.53	2.76_5	−8.46
7	51.0	28.2	42.7	23.6	1300	1.80	5.53	2.76_5	−8.54

[a] $\Delta H_p = Q_{exp}\, V^{-1}$, where Q_{exp} is the heat evolved during the experiment (μW) and V is the substrate flowrate (mol s^{-1} 10^{-9}).

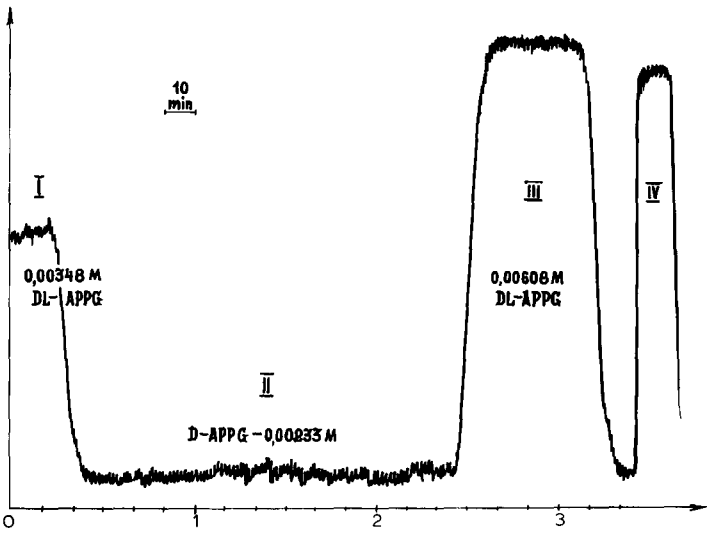


Fig. 1. Heat-evolution curves plotted during microcalorimetric experiments in which DL-APPG and D-APPG were used. (I) Experiment No. 1, Table 2; (II) experiment with D-isomer; (III) experiment No. 2, Table 2; (IV) electrical calibration.

The data, presented in Tables 1 and 2 relate to the following generalized reaction:

$$R_1CONHR_2 + H_2O = R_1COO^- + {}^+NH_3R_2 \quad (1)$$

where R_1 and R_2 are organic radicals which are both capable, in principle, of bearing a charge. The average enthalpy of reaction (1) for peptide-bond hydrolysis ($\Delta\overline{H}_r$), calculated from the data in Table 1, is $\Delta\overline{H}_r = -7.6 \pm 2.0$ kJ mol^{-1}.

As mentioned earlier [4], when a bulk of experimental data is available the researcher can infer not only the average enthalpy of hydrolytic reaction for a whole class of compounds (in this case, hydrolytic reactions in peptide bonds), but also the value of $\Delta\overline{H}_r$ for a limited number of compounds. The latter values of $\Delta\overline{H}_r$ will naturally differ less from the enthalpy of each individual reaction than the $\Delta\overline{H}_r$ for the entire class of reactions.

The data in Table 1 allow approximate average enthalpies for hydrolytic reactions in peptide-bonded compounds to be calculated. From the data in Table 1 we have calculated the $\Delta\overline{H}_r$ of hydrolysis for the tyrosine–glycine bond (-5.83 ∓ 0.65 kJ mol^{-1}) and that for tyrosine–benzoic acid (-8.81 ± 0.90 kJ mol^{-1}); in the latter case the maximum errors, specified in Table 1 for individual reactions, were assumed as $\Delta\overline{H}_r$ errors. Further $\Delta\overline{H}_r$ values for different types of peptide bonds can be obtained in a similar manner.

Thermochemical data, pertinent to hydrolytic reactions in different amide-bonded organic compounds, are plotted in Fig. 2. The figure illustrates the results of thermochemical studies on the hydrolytic reactions in glycyl-L-phenylalanineamide (**I**), benzoyl-L-tyrosineamide (**II**) and benzoyl-

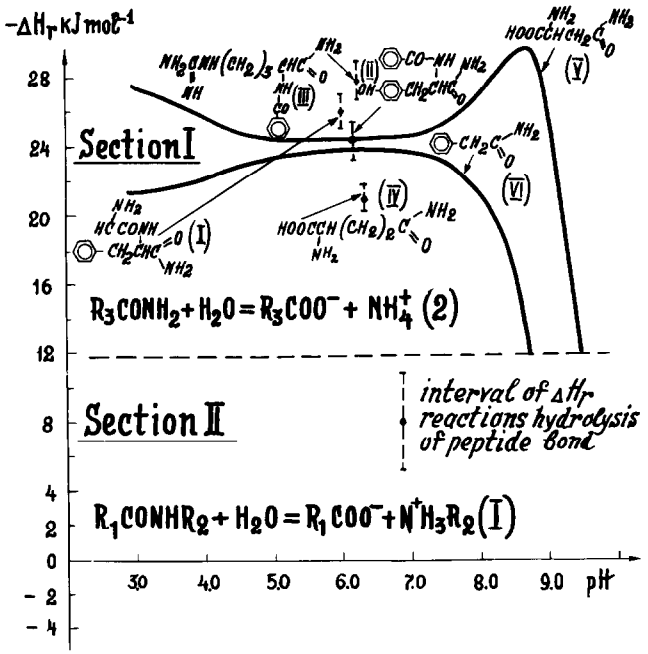


Fig. 2. Enthalpies of the hydrolytic reactions of various organic compounds containing amide (section I) and peptide (section II) bonds.

L-arginineamide (**III**) and in glutamine (**IV**), asparagine (**V**) and phenylacetamide (**VI**). Compounds **I–III** have been studied by Sturtevant and co-workers [6–10] and compounds **IV–VI** were studied by Kitzinger and Hems [12] and by the present authors [13].

With respect to asparagine and phenylacetamide, the enthalpies of the hydrolytic reactions were calculated for different pH values (Fig. 2), making use of the ionization enthalpies of the reaction components [13,14]. The enthalpies of the hydrolytic reactions of these amides are very much dependent on pH. However, when the pH is close to neutral, the following generalized equation holds

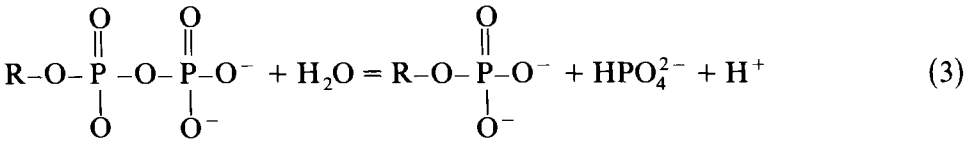
$$R_3CONH_2 + H_2O = R_3COO^- + NH_4^+ \quad (2)$$

For amide-bond hydrolytic reactions, the enthalpies of the hydrolytic reactions of different amides are close to one another (Fig. 2). On the basis of the experimental data available (Fig. 2), we found the $\Delta\overline{H}_r$ value for the amide bond hydrolytic reaction to be -24.7 ± 2.0 kJ mol^{-1}.

THERMOCHEMISTRY OF THE HYDROLYTIC REACTIONS OF ORTHOPHOSPHATE ESTERS

Orthophosphate esters, ATP, ADP, GTP and many others, participate in quite dissimilar biochemical reactions [1,2]. Dependable thermochemical

data, relevant to the hydrolysis reaction of these orthophosphates, were first obtained by Sturtevant and co-workers [15–17]. The enthalpies of the hydrolytic reactions of esters, containing two or more radicals of orthophosphoric acid (ATP, ADP, GTP, etc.) are given in Table 3. The thermochemical data (Table 3) relate the following generalized reaction



$$R\text{–}O\text{–}P(O)(O^-)\text{–}O\text{–}P(O)(O^-)\text{–}O^- + H_2O = R\text{–}O\text{–}P(O)(O^-)\text{–}O^- + HPO_4^{2-} + H^+ \quad (3)$$

where R is AMP^-, IMP^-, GMP^-, guanosine or hydrogen. The average $\Delta\overline{H}_r$ value, calculated with the data in Table 3, is -21 ± 3 kJ mol^{-1}.

The compounds under examination (ATP, ADP and GTP), generally function in living organisms as complexes with Mg^{2+} or other metal ions.

Thermodynamic parameters of complex formation (chelation) are cited in a number of works (see, for example, [19–21]). The $\Delta\overline{H}_{(3)}$ value and enthalpies of chelation of the compounds with metal ions can be used as a basis for calculating the enthalpies of hydrolytic reactions in certain complexes. For instance, the difference of enthalpies ($\Delta\Delta H_f$) for complex-forming ions of magnesium Mg^{2+} with ADP^{3-} and ATP^{4-} equals $\Delta\Delta H_f = \Delta H_f$ [(ADP) Mg]$^{-1} - \Delta H_f$[(ATP) Mg]$^2 = -5$ kJ mol^{-1} [22–24]. Accordingly, ΔH_r for the hydrolysis reaction of the [(ATP) Mg]$^{2-}$ complex, expressed by:

$$[(\Delta ATP)\ Mg]^{2-} + H_2O = [(ADP)\ Mg]^- + HPO_4^{2-} + H^+ \quad (4)$$

will be $\Delta H_4 = -26$ kJ mol^{-1}.

The hydrolysis enthalpies for 3′, 5′ and 2′, 3′ cyclic nucleotides, given in Table 4, differ substantially from those in Table 3 which are characteristic of non-cyclic nucleotides. The enthalpies of hydrolytic reaction of orthophosphates, which are not included in Tables 3 and 4, are cited in Table 5. The enthalpies of reaction, listed in Table 5, have been calculated by us from literature data [17,18,27–31] and from the enthalpy of the reaction [4]. The first five reactions (Table 5) proceeded as hydrolysis of compounds in which

TABLE 3

Enthalpies of hydrolytic reaction in esters containing two or more radicals of orthophosphoric acid

Compound	ΔH_r (298.15 K) (kJ mol^{-1})	Ref.
Adenosinetriphosphate (ATP^{4-})	-20 ± 3 (293 K)	15, 16
Adenosinetriphosphate (ATP^{4-})	-21	17
Inosinetriphosphate (ITP^{4-})	-20 (293 K)	16
Pyrophosphate ($HP_2O_7^{3-}$)	-21	17
Guanosinetriphosphate (GTP^{4-})	-23 ± 2	18
Guanosinediphosphate (GDP^{3-})	-23 ± 2	18

TABLE 4

Enthalpies of hydrolytic reactions in 3,5- and 2,3-nucleotides [25,26]

Compound	ΔH_r (298.15 K) (kJ mol^{-1})	$\Delta \bar{H}_r$ (kJ mol^{-1})
Cyclic 3′, 5′AMP	−59.0	−52.7
Cyclic 3′, 5′ GMP	−43.9	
Cyclic 3′, 5′ IMP	−56.1	
Cyclic 3′, 5′ dAMP	−54.4	
Cyclic 3′, 5′ UMP	−50.2	
Cyclic 2′, 3′AMP	−39.3	−36.4
Cyclic 2′, 3′ GMP	−39.7	
Cyclic 2′, 3′ CMP	−33.9	
Cyclic 2′, 3′ UMP	−32.6	

orthophosphoric acid is bonded to a carbohydrate radical. The first four reactions have similar hydrolysis enthalpies: $\Delta \bar{H}_r = +2.4$ kJ mol^{-1} (the fifth reaction is an exception: $\Delta H_r = -6.0$ kJ mol^{-1}). The enthalpies of hydrolysis reactions in AMP, IMP and other similar non-cyclic nucleotides, will probably be similar to the enthalpy average quoted above ($\Delta \bar{H}_r = +2.4$ kJ mol^{-1}).

Note also that the enthalpies of hydrolytic reaction in fructoso-6-phosphate and fructoso-1,6-diphosphate differ from each other by more than 10 kJ mol^{-1} (Table 5).

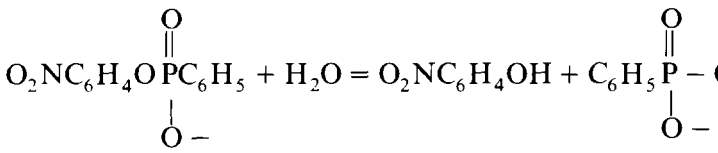
TABLE 5

Enthalpies of hydrolytic reactions in orthophosphate esters

No.	Reaction (kJ mol^{-1})	ΔH_r (298.15 K)	Ref.
1	Guanosine-monophosphate^{2-} + H_2O = guanosine + HPO_4^{2-}	+2.7	18
2	Glycerol-3-phosphate^{2-} + H_2O = glycerol + HPO_4^{2-}	+3.9	27
3	Glucoso-6-phosphate^{2-} + H_2O = glucose + HPO_4^{2-}	+2.0	28, 27
4	Mannoso-6-phosphate^{2-} + H_2O = mannose + HPO_4^{2-}	+0.9	29
5	Fructoso-6-phosphate^{2-} + H_2O = fructose + HPO_4^{2-}	−6	29
6	Fructoso-1.6-diphosphate^{4-} + H_2O = fructoso-6-phosphate^{2-} + HPO_4^{2-}	−17	27, 31
7	Creatinephosphate^{3-} + H_2O = creatine^{-} + HPO_4^{2-}	−44	27
8	Phosphoenolpyruvate^{3-} + H_2O = pyruvate^{-} + HPO_4^{2-}	−33	30, 27
9	*p*-Nitrophenylphosphate^{-} + H_2O = *p*-nitrophenol + HPO_4^{2-} + H^+	−22	17

The enthalpies of hydrolytic reaction in creatinephosphate and phosphoenolpyruvate (reactions 7 and 8 in Table 5) are similar quantitatively to the enthalpies of hydrolytic reactions in cyclic nucleotides (Table 4), while those in *p*-nitrophenylphosphate (reaction 9, Table 5) are similar to the enthalpies of hydrolytic reaction in non-cyclic nucleotides (Table 3).

We shall conclude this part of this article by giving (for comparison) the enthalpy of hydrolytic reaction in *p*-nitrophenyl-phenylphosphate [35].



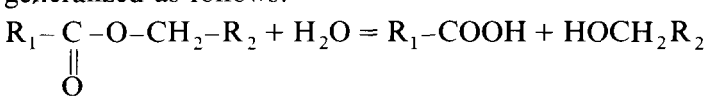
$$O_2NC_6H_4O\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}C_6H_5 + H_2O = O_2NC_6H_4OH + C_6H_5\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}} - OH \qquad (5)$$

$\Delta H_5 = -29.7$ kJ mol^{-1}.

The ΔH_5 value is similar to the enthalpy of hydrolytic reaction of *p*-nitrophenylphosphate, if an uncharged molecule of n-nitrophenol and $H_2PO_4^-$ are considered as end products. In this case, the enthalpy of hydrolytic reaction of n-nitrophenylphosphate equals -26.3 kJ mol^{-1} [17].

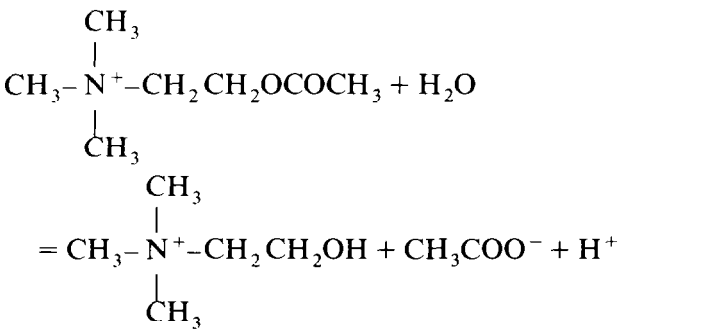
THERMOCHEMISTRY OF HYDROLYTIC REACTIONS IN COMPOUNDS, CONTAINING ESTER AND GLUCOSIDE BONDS

The hydrolysis reaction for ester-bond containing compounds (except esters containing inorganic acids, for instance orthophosphoric acid) can be generalized as follows:



$$R_1-\overset{\overset{O}{\|}}{C}-O-CH_2-R_2 + H_2O = R_1-COOH + HOCH_2R_2 \qquad (6)$$

where R_1 and R_2 are organic radicals. The organic ester compounds, having structures $C_2H_5OCOCH_3$ and $CH_3CONHCH_2CH_2OCOCH_3$, were studied thermochemically by Wadso [32]. In these compounds, according to eqn. (6), the enthalpies of hydrolysis reactions will be 1.80 ± 0.25 and 1.97 ± 0.30 kJ mol^{-1}, respectively. The enthalpy of hydrolytic reaction for *N*-acetylphenylalanine methylether $\Delta H_r = 1.63 \pm 0.25$ kJ mol^{-1}, obtained by the authors [11], agrees with Wadso's data [32], within admissible errors. Sturtevant [33] found the enthalpy of hydrolytic reaction for acetylcholine



$$CH_3-\overset{\overset{CH_3}{|}}{\underset{\underset{CH_3}{|}}{N^+}}-CH_2CH_2OCOCH_3 + H_2O$$

$$= CH_3-\overset{\overset{CH_3}{|}}{\underset{\underset{CH_3}{|}}{N^+}}-CH_2CH_2OH + CH_3COO^- + H^+ \qquad (7)$$

to be -3.57 ± 0.05 kJ mol^{-1} in phosphate buffer solution. Having interpreted this value for a non-dissociated state on the basis of phosphate buffer ionization enthalpy ($\Delta H_i = -4.10 \pm 0.2$ kJ mol^{-1}) and acetic acid ionization enthalpy ($\Delta H_i = -0.3 \pm 0.1$ kJ mol^{-1}) [14], we obtained $\Delta H_7^{\text{non-diss}} = 0.85 \pm 0.3$ kJ mol^{-1}. In addition to the above four compounds, Shyamada et al. [34] studied *N*-acetyl-L-tryptophan ethyl ether. The enthalpy of hydrolytic reaction for this compound has been found from eqn. (6) to be 2.5 ± 2.1 kJ mol^{-1}, assuming the enthalpy of the phosphate buffer solution ionization to be -4.10 ± 0.2 kJ mol^{-1}.

Thus, on the grounds of the experimental data cited above, the average value of enthalpy for the hydrolytic reaction in ester bonds is 1.8 kJ mol^{-1}, compared with the enthalpy of the hydrolytic reaction in the thioether bond (in non-dissociated state) which is -3.8 kJ mol^{-1} [32].

Original reports on the thermochemistry of hydrolytic reactions in glucoside bonds have been discussed in detail in the review by Ono and Takahashi [36]. Therefore, we shall only note here that the average enthalpy of hydrolytic reaction in the $\alpha - 1.4$ glucoside bond is -4.6 kJ mol^{-1}, and in the $\alpha - 1.6$ glucoside bond $+5.3$ kJ mol^{-1}.

CONCLUSION

It has been shown that the averaging method is applicable to the calculation of enthalpies for various types of hydrolytic enzymatic reactions. On the basis of literature data and from our own experiments, average enthalpies of hydrolysis reactions have been obtained for compounds, containing peptide, amide, ester bonds and orthophosphates.

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