Journal of Thermal Analysis, Vol. 54 (1998) 311-315

THERMODYNAMICS OF PROTOLYTIC EQUILIBRIA IN AQUEOUS SOLUTIONS OF BIOLOGICALLY ACTIVE LIGANDS

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Abstract

The protolytic equilibria in aqueous solutions of different amino acids and dipeptides were studied by a direct calorimetric method at 288–328 K. The measurements were made with an isothermal-shell calorimeter with automatic temperature recording. The thermodynamic characteristics of the processes of acid-base equilibria were found from the combined results of thermochemical measurements and equilibrium constant data. Gurney concepts were used to analyse the thermodynamic characteristics of reactions. Comparisons of the temperature-dependent and temperature-independent contributions to the Gibbs energy and enthalpy change afford the criteria of the variability in sign of the ΔH of the process, i.e. they allow prediction of the magnitude of the temperature θ at which $\Delta H = 0$.

Keywords: acid-base equilibria, amino acid, enthalpy, entropy, peptide

Introduction

Amino acids and low-molecular peptides play an important role in biochemical processes as they are structural components of proteins and other biologically important substances. However, information on the thermodynamic properties of these compounds is insufficient, and dissociation heat data on many dipeptides are not available at all.

Thermochemical investigations of the equilibria of α- and β-alanine, glycyl-γ-aminobutyric acid, *D,L*-α-alanyl-*D,L*-α-alanine, glycyl-*L*-asparagine and others in aqueous solutions have been carried out in our laboratory.

The difference in the stepwise dissociation constants of the amino acids and peptides by a factor of 4–7 allowed the independent determination of the stepwise ionization heats.

Experimental

Amino acids and peptides produced by Reanal in 'chromatographically pure' quality were used in the present work. The heat effects were measured with the

1418–2874/98/ $ 5.00

Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

isothermal-shell calorimeter, with automatic registration of the experimental calorimetric curve [1]. The calorimeter was calibrated by means of electric current. KNO_3, $LiNO_3$ and $NaNO_3$ were used as background electrolytes. Measurements were made in the temperature range 288–323 K at ionic strengths in the interval 0.5–1.5. The equilibrium compositions of the solutions under the various experiment conditions were calculated by means of the RRSU program on a PC 486 DX2 [2].

The reliable literature data on the acid and base dissociation constants of the above amino acids and peptides allow an interpretation of the calorimetric measurement results with appropriate accuracy.

Two independent procedures were used to determine the stepwise dissociation heats of the amino acids and peptides.

The heat effects of the interactions of the mineral acid solution (HNO_3) with the peptide (amino acid) solutions were measured in the pH interval 3.6–2.8, together with the heats of HNO_3 dilution in the background electrolyte solutions. The calculations showed that 60–80% of the HNO_3 placed in the ampoule reacted. In the second procedure, a peptide (amino acid) solution with a concentration of 0.5–0.9 mol kg^{-1} was placed in the ampoule, and HNO_3 solution was used as the calorimetric liquid. The heat effects were found as the differences between the experimentally measured mixing heats and the heats of dilution of the peptide (amino acid) solutions in the supporting electrolyte solution. In the determinations of the dilution heats, the pH of the electrolyte solution was found to be equal to the original pH of the peptide (amino acid) solution; thus, dilution proceeded without change in the equilibrium composition of the system.

In the determinations of the zwitterion base dissociation heats, the interaction heats of the peptide (amino acid) solutions located in the ampoule with KOH solution were measured. The interaction heats of HNO_3 solution with the peptide solution in the pH interval 9–7 were determined by the second procedure. The corresponding dilution heats were measured, too.

Results and discussion

The dissociation heats of the peptides and the amino acids in the standard solution were found by extrapolation the dissociation heat to zero ionic strength by means of an equation with a single parameter [3]:

$$\Delta H - \Delta z^2\Psi(I) = \Delta H^0 + iI \qquad (1)$$

where ΔH and ΔH^0 are the enthalpy changes at the final ionic strength and at $I=0$, respectively; $\Psi(I)$ is a theoretically calculated function dependent on the ionic strength; and i is an empirical coefficient.

The thermodynamic characteristics of the stepwise dissociation processes of the amino acids and peptides were found by using both the results of the thermochemical measurements and the equilibrium constant data.

Table 1 Standard thermodynamic characteristics of the acid-base interaction processes of amino acids and peptides in aqueous solution and their temperature-dependent and temperature-independent enthalpy constituents

Process	T/ K	pK°	Δ_rH°/ J mol^{-1}	$-\Delta_rS$°/	$-\Delta_rC_p^{\circ}$/	$-\Delta_rH_{dep}$/	Δ_rH_{indep}/
				J mol^{-1} K^{-1}		J mol^{-1}	
H_2Ala^+=	298	2.34±0.04	3400±130	35.2±0.9	130±10	5400	8800
$HAla^{\pm}+H^+$	308	2.34±0.04	2100±110	39.4±0.9		6500	8600
	318	2.34±0.04	900±130	43.0±0.9		7700	8600
	328	2.34±0.04	−500±100	45.8±0.9		9100	8600
$HAla^{\pm}$=	298	10.1±0.04	45500±270	40.4±1.2	50±20	6000	51500
Ala^-+H^+	308	9.83±0.04	45100±340	42.0±1.4		6400	51500
	318	9.59±0.04	44400±340	44.0±1.4		7100	51500
D,L-α-Ala-*D,L*-	288	3.16±0.02	−510±210	62.2±1.1	100±10	6520	6010
-α-$AlaH_2^+$ =	298	3.16±0.02	−1720±110	66.3±1.2		7790	6070
D,L-α-Ala-*D,L*-	308	3.17±0.02	−2440±120	68.6±0.8		8990	6550
-α-$AlaH^{\pm}$ + H^+							
D,L-α-Ala-*D,L*-	288	8.83±0.03	45690±720	10.4±3.1	0	2980	48670
-α-$AlaH^{\pm}$ =	298	8.55±0.03	45890±560	9.8±2.4		3370	49260
D,L-α-Ala-*D,L*-	308	8.29±0.03	45690±700	10.4±2.8		3860	49550
-α-Ala^- + H^+							
Gly-γ-$AbuH_2^+$=	298	4.22±0.02	1640±60	76.0±0.2	150±2	8940	10130
Gly-γ-$AbuH^{\pm}+H^+$	308	4.22±0.02	430±200	79.3±0.3		9940	10370
	318	4.22±0.02	−1330±110	84.9±0.1		11620	10290
Gly-γ-$AbuH^{\pm}$=	298	8.34±0.01	42620±450	16.7±1.3	0	3920	46540
Gly-γ-Abu^-+H^+	308	8.10±0.01	42430±540	17.3±1.6		4470	46900
	318	7.87±0.01	42250±450	17.9±1.2		5030	47280
Gly-*L*-$AsnH_2^+$=	288	3.07±0.02	1580±200	53.2±0.5	100±10	5900	7480
Gly-*L*-$Asn^{\pm}+H^+$	298	3.06±0.02	650±140	56.7±0.5		7020	7670
	308	3.06±0.02	−340±150	59.7±0.3		8200	7860
Gly-*L*-$AsnH^{\pm}$=	288	8.96±0.03	45620±480	13.2±1.1	40±30	3180	48800
Gly-*L*-Asn^-+H^+	298	8.69±0.03	45210±520	14.7±1.2		3750	48960
	308	8.43±0.03	44850±430	15.8±0.8		4340	49190

The standard thermodynamic characteristics of the processes of acid-base interaction for the amino acids and peptides in aqueous solution are given in Table 1. It can be seen that in the temperature range 288–318 K the standard heat of carboxyl group ionization for the investigated peptides changes sign. The temperature θ at which the heat effect changes sign could be evaulated via the equation

$$\theta = 298.15 - \frac{\Delta H_{298.15}}{\Delta c_{\mathrm{p}}} \qquad (2)$$

The positions of the carboxyl and amino groups in the amino acids and peptides influence the magnitude of the entropy change during dissociation. The greater the distance of separation of the negative and positive charge carriers in the zwitterion, the higher is its hydration degree. Indeed, the process of carboxyl group dissociation in the peptides as compared with that in the amino acids where the zwitterion formation takes place is accompanied by a higher absolute value of the entropy change ($\Delta_r S^\circ_{1(\mathrm{Ala-Ala})} = -66.3\pm1.2$ J mol^{-1} K^{-1}, $\Delta_r S^\circ_{1(\mathrm{Ala})} = -35.2\pm0.9$ J mol^{-1} K^{-1}) [4].

For the magnitude of $\Delta_r S$ which characterizes the carboxyl group dissociation in peptides with different structures, the same regularity is true. Accordingly, for the carboxyl group dissociation of glycyl-γ-aminobutyric acid, the entropy change is greater in absolute magnitude ($\Delta_r S^\circ_{1(\mathrm{Gly-gamma-Abu})} = -76.0\pm0.2$ J mol^{-1} K^{-1}) [5] than the analogous changes for carboxyl group dissociation in *D,L*-α-alanyl-*D,L*-α-alanine and glycyl-α-asparagine.

The numeral values of the temperature-dependent and temperature-independent enthalpy constituents are given in Table 1. The technique for calculation of these is reported elsewhere [6]. The distinction of $\Delta_r H^\circ_{\mathrm{indep.}}$ which characterizes the energies of proton interaction with nitrogen and oxygen-containing groups deserves attention. In the former case, dissociation of the betaine proton of amino acids and peptides is characterized by significant values of $\Delta_r H^\circ_{\mathrm{indep.}}$, whereas $\Delta_r H^\circ_{\mathrm{indep.}}$ is not high for dissociation of a proton connected with an oxygen atom.

Some regularities may be observed as concerns the influence of temperature on the dissociation heats of different functional groups of biologically active ligands. In the splitting-off of a proton connected with the nitrogen atom of an amino acid or peptide, the temperature-independent enthalpy constituents are much larger in absolute value than the contribution of the temperature-dependent constituents. For dissociation of the carboxyl group proton, the temperature-dependent and temperature-independent enthalpy constituents are very close to each other in magnitude. In this case, ΔC_{p} is of the order of -100 J mol^{-1}K^{-1}. This value is also characteristic of the process of amino acid carboxyl group dissociation.

The standard enthalpies of formation of amino acids and peptides were calculated from the experimental values of heats of dissolution, acid-base reactions and combustion of the investigated compounds [7].

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