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THE THERMODYNAMICS OF IONISATION OF GLYCINE IN METHANOL + WATER MIXTURES AND THE DETERMINATION OF SINGLE ION THERMODYNAMICS

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ABSTRACT

In order to comprehend the effect of solvents on the thermodynamic parameters of amino acids, the thermodynamic dissociation constants K_1 and K_2 for the reactions

$$RH_2^+ + H_2O \rightleftharpoons H_3O^+ + RH^\pm \qquad K_1 = \frac{a_{H_3O^+} \cdot a_{RH^\pm}}{a_{RH_2^+}} \tag{1}$$

and

$$RH^\pm + H_2O \rightleftharpoons H_3O^+ + R^- \qquad K_2 = \frac{a_{H_3O^+} \cdot a_{R^-}}{a_{RH^\pm}} \tag{2}$$

have been determined in methanol + water mixtures pH-metrically. The measurements were carried out in dilute solutions and in the absence of neutral electrolytes to minimize the "salt effect" as far as practicable so that the "medium effects" on the dissociation constants of the amino acid could be properly understood.

The enthalpy values for reactions (1) and (2) (up to 44.14% of methanol) have been determined calorimetrically. It has been observed that the conversion of amino acids into cations and anions is favourable both from enthalpic and entropic considerations so that the reverse reactions of (1) and (2) are spontaneous. The thermodynamic properties of glycine have been explained in terms of hydrophobic and hydrophilic interactions of glycine with solvent mixtures.

In order to gain better insight into the nature of specific solute–solvent interactions, we have analysed the results in terms of single ion values determined using thermodynamic values from the present work and relevant data from other work in our laboratory.

INTRODUCTION

The thermodynamic parameters of different acid–base equilibria in mixed and non-aqueous solvents are relatively few, though these values are of great help in understanding the various factors associated with solute–solvent interactions.

The unique behaviour of amino acids and their usefulness in maintaining a harmonious equilibrium between synthesis and decay in the living world

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make them a distinct class of compounds. The "dipolar" nature of amino acids makes their behaviour different from organic aliphatic (or aromatic) acids or amines. Therefore, studies of the thermodynamic parameters of amino acids in different mixed or non-aqueous solvents are of great relevance in understanding the thermodynamic behaviour of proteins.

The study of dissociation of amino acids and peptides in different mixed solvents is of great theoretical and practical interest and has attracted considerable attention [1–4]. Recently, Lahiri and co-workers [5,6] have undertaken systematic studies on the dissociation constants of amino acids

$$RH_2^+ + H_2O \rightleftharpoons H_3O^+ + RH^\pm \qquad (1)$$

$$RH^\pm + H_2O \rightleftharpoons H_3O^+ + R^- \qquad (2)$$

where $RH^\pm$ represents different amino acids, in different mixed solvent media.

We determined the first and second dissociation constants of glycine in different methanol + water mixtures pH-metrically. We also determined enthalpies of ionisation of glycine at 298 K in methanol + water mixtures (0–44.1 wt% of methanol) calorimetrically.

The results of our investigations are presented to this communication.

EXPERIMENTAL

Glycine (GR, Merck) was dried at 383 K for 1 h and kept in a desiccator. Methanol (AR, BDH) was distilled before use and the middle fraction was collected and utilised within 24 h. Perchloric acid and caustic soda (GRE, Merck) were standardized in the usual way. The solutions were made with double-distilled water from an all-glass distilling apparatus.

The weight percentages of the organic solvent were determined in the usual way [7]. The dielectric constant values were taken from data in the literature [8].

pK_1 and pK_2 values of glycine were determined as previously described [5,6]. These values are presented in Table 1.

Details of the calorimeter used in the present investigation have been described earlier [9,10]. For measurements of heat of neutralisation (ΔH) corresponding to the neutralisation of the basic group of amino acids (reaction 1), 250 ml of $HClO_4$ solution ($\sim$ 0.5 M) in appropriate solvents were taken in a reaction flask. In glass bulbs, a definite volume of the desired amino acid (5 ml of 3.03–6.33 M) was taken in an appropriate solvent, to avoid heat change due to mixing. After proper equilibration at the desired temperature, the bulb was broken and the heat change was measured by the change in resistance, calibrated against a known heat supplied before each set of measurements. Suitable blank titrations were performed. The values

TABLE 1

pK_1 and pK_2 values of glycine, the heats of neutralisation of glycine by alkali, $\Delta H^0_{\mathrm{neut}}$ (kJ mol^{-1}), and the thermodynamics of glycine (RH$^{\pm}$) and those for reactions (1) and (2) in methanol + water mixtures at 298 K (kJ mol^{-1})

Wt% of methanol	pK_1	pK_2	ΔG^0 (RH$^{\pm}$)	ΔH^0 (RH$^{\pm}$)	$T\Delta S^0$ (RH$^{\pm}$)	$\Delta H^0_{\mathrm{neut}}$	ΔG^0_1	ΔH^0_1	$T\Delta S^0_1$	ΔG^0_2	ΔH^0_2	$T\Delta S^0_2$
0.0	2.34 (±0.01)	9.67 (±0.02)	−2.98	6.54	9.52	−13.72 (±0.10)	13.42 (±0.05)	6.59 (±0.10)	−6.83	55.18 (±0.12)	51.87	−3.31
8.0	2.55 (±0.01)	9.70 (±0.02)	−2.86	7.50	10.36	−8.45 (±0.08)	14.74 (±0.06)	8.99 (±0.10)	−5.75	55.36 (±0.12)	44.74	−10.62
16.4	2.66 (±0.02)	9.72 (±0.02)	−2.74	8.61	11.35	−4.81 (±0.04)	15.09 (±0.12)	10.29 (±0.10)	−4.80	55.47 (±0.12)	45.38	−10.09
25.2	2.70 (±0.02)	9.76 (±0.03)	−0.77	10.21	10.98	−2.43 (±0.15)	15.26 (±0.12)	11.68 (±0.04)	−3.58	55.70 (±0.20)	44.42	−11.28
34.4	2.88 (±0.02)	9.82 (±0.03)	−0.50	13.95	14.45	1.72 (±0.04)	16.63 (±0.12)	12.05 (±0.04)	−4.58	56.04 (±0.20)	44.56	−11.48
44.1	3.05 (±0.02)	9.85 (±0.03)	0.13	5.94	5.61	4.18 (±0.12)	18.24 (±0.12)	13.15 (±0.04)	−5.09	56.21 (±0.20)	43.32	−12.39

thus obtained were subtracted from or added to the heat of neutralisation data.

For measurement of heat of neutralisation of the acid group of the amino acid, corresponding to reaction (2), the process was the same, only $HClO_4$ was replaced by NaOH (~ 0.1 M).

RESULTS

The dissociation constants for reactions (1) and (2) can be represented by

$$K_1 = \frac{C_{H^+}C_{RH^\pm}}{C_{RH_2^+}} \frac{f_{H^+}f_{RH^\pm}}{f_{RH_2^+}}$$

and

$$K_2 = \frac{C_{H^+}C_{R^-}}{C_{RH^\pm}} \frac{f_{H^+}f_{R^-}}{f_{RH^\pm}}$$

Values of K_1 and K_2 were determined as described earlier [5,6]. The pK_1 values obtained are in good agreement with those reported earlier [5]. In calculating K_2, we have taken into consideration the values of the autoprotolysis constants of water in methanol + water mixtures from the literature [11]. The measurements were carried out in dilute solutions and in the absence of neutral electrolytes to minimize the "salt effect" as far as practicable [12] so that the "medium effect" on the dissociation constants of the amino acid could be properly understood. Moreover, in dilute solutions, the activity coefficients of the "zwitterion" and other ions have been taken to be unity.

The enthalpy changes for reactions (1) and (2) were calculated using the relation

$$\Delta H = \frac{Q}{X} \text{Joules}$$

where Q is the heat liberated (Q negative) or absorbed (Q positive) to neutralise X g mol l^{-1} of amino acid. It has been found that heat is always liberated due to the addition of acid or alkali to the amino acid.

We have used a fairly dilute solution of amino acids and variations of ΔH values have been found to be within the limits of experimental error. Since amino acids are completely neutralised by the acid or alkali, the experimental ΔH value can be taken as ΔH^0. However, the addition of alkali means that the enthalpy is changed due to neutralisation of H^+ ion and the enthalpy change (ΔH_2^0) for reaction (2). Thus, $\Delta H_2^0 + \Delta H^0_{H_2O(\text{in water or mixed solvents})} = \Delta H^0_{\text{obs.}}$. $\Delta H^0_{H_2O(\text{in mixed solvents})}$ values were interpolated from data in the literature [11]. The heats of neutralisation of glycine in different methanol–water mixtures are shown in Table 1.

ΔS_1^0 and ΔS_2^0 values for reactions (1) and (2) have been calculated using ΔG_1^0 and ΔG_2^0 values.

The thermodynamic properties of glycine are recorded in Table 1.

DISCUSSION

The pK_1 value of glycine has been observed to increase with increasing alcohol contents of the mixture but the magnitude of this increase is much smaller than those of the corresponding carboxylic acid due to the "dipolar character" of the acid. However, pK_2 is relatively insensitive to solvent changes, though a slight increase in pK_2 has been observed at higher percentages of alcohol.

The enthalpy changes for reactions (1) and (2) for glycine are 6.59 and 51.87 kJ mol^{-1}, respectively. They are not in agreement with those (4.84 and 45.21 kJ mol^{-1}) obtained from temperature coefficient measurements reported in the literature [4]. The disagreement appears to be considerable. However, calorimetric calculations are expected to give better results compared to values obtained from temperature coefficient measurements. It is to be noted that the heat of neutralisation of glycine (13.78 kJ mol^{-1}) by NaOH in water compares well with that (12.18 kJ mol^{-1}) reported by Goudard et al. [13].

It is apparent that the conversion of amino acid into cations and anions is favourable both from enthalpic and entropic considerations so that the backward reaction is spontaneous. This is probably the reason why the amino acids are easily converted into peptides or proteins. Due to the "zwitterionic" nature of the amino acid, glycine is solvated firmly by a large number of water molecules. Thus, the formation of cations or anions by acid or alkali involves the liberation of immobilized solvent molecules leading to an increase in entropy and the liberation of energy.

Table 1 shows that ΔG_1^0 and ΔH_1^0 values increase continuously whereas ΔS_1^0 values increase and become maxima between 24 and 34 wt% of methanol.

The ΔH_2^0 and ΔS_2^0 values pass through a minimum at about 8 wt% of methanol. However, it should be noted that, due to limitations described above, the error range for determining ΔG_2^0, ΔH_2^0 and ΔS_2^0 would be considerably higher.

Enthalpy and entropy changes are generally attributed to structural changes associated with the introduction of methanol to water. It is well known that the addition of methanol first strengthens the three-dimensional water structure, and passes through a maximum at about 0.2–0.3 mole fraction of methanol, after which structural collapse begins. Methanol + water mixtures show a maximum in exothermic enthalpy of mixing at about $X_2 \approx 0.2$, a minimum in $T\Delta S$ at $X_2 = 0.3$ and a maximum in the positive free energy of mixing at $X_2 \approx 0.5$ (X_2 = mole fraction of alcohol) [14]. It is expected that structural changes of the solvent mixtures may well be reflected in the enthalpy and entropy values of the amino acids as presented in Table 1, whereas the free energy changes are governed by electrostatic changes. However, a clear correlation between thermodynamic properties and solvent properties could not be achieved. Glycine with two polar groups and an apolar $-CH_2-$ group will undergo hydrophilic and hydrophobic interactions with solvent molecules. It is hydrated by a large number of water molecules [15] but as methanol is introduced, hydrophobic interactions increase and solvational nature changes. Moreover, the ratio of zwitterion to neutral molecules will change but the change will be too small to affect the result to a great extent.

In order to gain better insight regarding the nature of solute–solvent interactions, it is desirable to analyse the results in terms of single ion values.

The thermodynamics of single ions can be derived using the following relations. For reaction (1), we have

$$\Delta X_t^0(1) = \Delta X_t^0(H^+) + \Delta X_t^0(RH^\pm) - \Delta X_t^0(RH_2^+)$$

or

$$-\Delta X_t^0(RH_2^+) = \Delta X_t^0(1) - \Delta X_t^0(H^+) - \Delta X_t^0(RH^\pm)$$

similarly, for reaction (2), we have

$$\Delta X_t^0(R^-) = \Delta X_t^0(2) - \Delta X_t^0(H^+) + \Delta X_t^0(RH^\pm)$$

where X represents the appropriate thermodynamic parameter, i.e., H, G and S.

$\Delta G_t^0(1)$ and $\Delta G_t^0(2)$ values have been taken from the present work. $\Delta G^0(RH^\pm)$ and $\Delta H^0(RH^\pm)$ values in water and mixed solvents were obtained from determinations of solubility and temperature coefficient measurements of the solubility of glycine determined in our laboratory [16].

The values of $\Delta G^0(RH^\pm)$, $\Delta H^0(RH^\pm)$ and $T\Delta S^0(RH^\pm)$ are given in Table 1. The $\Delta H_t^0(H^+)$ values have been determined utilising enthalpy values of the "isoelectric" reaction (A)

$$HPhen^+ \rightleftharpoons H^+ + Phen \qquad (A)$$

as reported earlier [17].

However, these values were recalculated to give the correct values. $\Delta H_t^0(H^+)$ values have also been recently reported by Abraham et al. using the $(Ph_4P^+, Ph_4As^+) = Ph_4B^-$ assumption [18]. $\Delta H_t^0(H^+)$ values calculated by us agree well (except at 16 wt%) with those reported by Abraham et al. if we consider the limitations of the different methods of calculation. $\Delta G_t^0(H^+)$ values have been taken from our earlier works [19]. $\Delta H_t^0(H^+)$ and $\Delta S_t^0(H^+)$ values are given in Table 2.

We have also calculated the single ion values (cation and anion of glycine) in water using $\Delta G^0(H^+)$ and $\Delta H^0(H^+)$ values from the literature [20] (after

TABLE 2

Thermodynamics of transfer of H^+, RH_2^+ (glycine cation) and R^- (glycine anion) from water to methanol + water mixtures at 298 K (kJ mol^{-1}) (Values in parentheses are based on $\Delta H_t^0(H^+)$ values from ref. 18)

Wt% of methanol	$\Delta G_t^0(H^+)$	$\Delta H_t^0(H^+)$	$T\Delta S_t^0(H^+)$	$\Delta G^0_{t(RH_2^+)}$	$\Delta H^0_{t(RH_2^+)}$	$T\Delta S^0_{t(RH_2^+)}$	$\Delta G_t^0(R^-)$	$\Delta H^0_{t(R^-)}$	$T\Delta S^0_{t(R^-)}$
8.0	−0.6	1.3	1.9	−1.8	−0.2	1.6	0.8	−7.5	−8.3
		(1.3)			(−0.1)	(1.7)		(−7.5)	(−8.3)
16.4	−1.3	8.8	10.1	−2.7	7.2	9.9	1.8	−13.2	−15.0
		(1.3)	(2.6)		(−0.3)	(2.4)		(−5.8)	(−7.6)
25.2	−1.8	5.0	6.8	−1.4	3.6	5.0	4.5	−8.8	−13.3
		(2.4)	(4.2)		(1.0)	(2.4)		(−6.0)	(−10.5)
34.4	−2.1	1.2	3.3	−2.9	3.2	6.1	5.4	−1.1	−6.5
		(5.2)	(7.3)		(7.2)	(10.1)		(−5.1)	(−10.5)
44.1	−3.2	−0.20	3.0	−4.9	−7.6	−2.7	7.2	−9.2	−16.4
		(6.3)	(9.5)		(−1.1)	(3.8)		(−15.7)	(−22.9)

$\Delta G^0(H^+)$ in water = −1087.4 kJ mol^{-1}
$\Delta H^0(H^+)$ in water = −1083.2 kJ mol^{-1}
$\Delta S^0(H^+)$ in water = 14J K^{-1} mol^{-1}

$\Delta G^0(RH_2^+)$ in water = −1103.7 kJ mol^{-1}
$\Delta H^0(RH_2^+)$ in water = −1096.3 kJ mol^{-1}
$\Delta S^0(RH_2^+)$ in water = 24.8J K^{-1} mol^{-1}

$\Delta G^0(R^-)$ = 1145.6 kJ mol^{-1}
$\Delta H^0(R^-)$ in water = 1128.5 kJ mol^{-1}
$\Delta S^0(R^-)$ = −57.4 J K^{-1} mol^{-1}

proper conversion into the molar scale) and other experimental values determined by us.

Values of the thermodynamics of transfer from water to methanol + water mixtures for ions are recorded in Table 2.

The single ion values in water show that the formation of the cation is favourable, usually accompanied by exothermic enthalpy changes and positive entropy changes, whereas the formation of the anion is energetically unfavourable, accompanied by endothermic enthalpy changes and negative entropy changes. The amino acid, due to its zwitterionic nature, is highly solvated due to electrostriction of the solvent molecules by the ionic charges. Formation of the cation leads to an increase in entropy due to the liberation of bound solvent molecules by the carboxylate ion and solvated H^+ ions. However, the formation of the anion is accompanied by a decrease in entropy due to the formation of negative ions and highly solvated H^+ ions.

The free energy of transfer of the cation passes through a maximum of about 24 wt% of methanol whereas the enthalpy of transfer is slightly exothermic initially, and passes through an endothermic maximum to become exothermic at 44 wt%. The entropy of transfer is positive for the cation but becomes negative at 44 wt% of methanol. The free energy of transfer of the anion is increasingly positive resulting from the favourable enthalpy of transfer but unfavourable entropy of transfer. The enthalpy and entropy appear to be maximum around 34 wt% of methanol. However, the comparison of the results is difficult as relatively little is known about the single ion values.

The results show that the enthalpies of transfer for the cation RH_2^+ closely resemble the enthalpies of transfer for Na^+, K^+ and Rb^+ [20] from water to methanol + water mixtures which show a decrease at the beginning, increase with increasing methanol content, and a decrease again from 40 wt% of methanol onwards. The enthalpies of transfer of the R^- ion, however, differ qualitatively from those of negative ions like Cl^-, Br^-, etc.

Fluctuations of the enthalpy of transfer and entropy of transfer are, however, quite expected in view of the structural variations of the solvent mixtures.

We do not like to infer much from these data, however. It is difficult to predict the errors involved in the determination of single ion values as it is difficult to predict the errors involved in the evaluation of $\Delta H_t^0(H^+)$ or $\Delta G_t^0(H^+)$.

It is imperative that we collect more data on single ion thermodynamics for a clear understanding of the nature of ion–solvent interactions and related solvation phenomena.

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