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Enthalpies of dilution of glycine, L-alanine and L-serine in aqueous potassium chloride solutions

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Abstract

The dilution enthalpies of glycine, L-alanine and L-serine in aqueous potassium chloride solutions of various molalities have been determined using LKB-2277 flow microcalorimetry at 298.15 K. The homogeneous enthalpic interaction coefficients over the whole range of aqueous potassium chloride solutions investigated have been calculated according to the excess enthalpy concept. It is found that pairwise enthalpic interaction coefficients *h*₂ of glycine and L-serine are all negative and become less negative with increasing of the molalities of potassium chloride, while pairwise enthalpic interaction coefficients h_2 of L-alanine are positive on which the influence of potassium chloride is not obvious. The results are interpreted from the point of view of solute–solute interactions involved by solvent effects. © 2004 Elsevier B.V. All rights reserved.

Keywords: Glycine; L-alanine; L-serine; Potassium chloride; Dilution enthalpy; Structural interactions; Electrostatic interactions

1. Introduction

The study of the thermodynamic stability of the native structure of proteins has proved quite challenging and still remains a subject of extensive investigation [1]. Amino acids and peptides are used as probe molecules to understan[d the](#page-6-0) complex nature of proteins. There is information on the zwitterionic nature of amino acids in water in the literature [2–5]. The properties of proteins**,** such [as t](#page-6-0)heir structure, solubility, denaturation, activity of enzymes, are greatly influenced by electrolytes [6,7]. The property of electrolyte known as structure-maker or structure-breaker has b[een wi](#page-6-0)dely used to understand the effect of electrolytes on the structure and function of both proteins and nucleic acids [3–10].

In [biolog](#page-6-0)ical fluids of living organisms, there contains a specified quantity of ions, especially sodium, potassium and chloride ions, which are indispensable for the metabolic processes of living organism to [proceed](#page-6-0) [11]. Information is available on activity coefficients, enthalpies and heat capacities of aqueous amino acids in electrolytes [12–18]. But most of the research works are focus on the dissolution

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or mixing enthalpies between amino acids and electrolytes [19–22]. A survey of the literatures indicates a lack of experimental data of the dilution enthalpies of amino acid in aqueous electrolyte solutions. Glycine is the most simple amino acid in nature. L-serine is the amino acid with polar side-chain – $CH₂OH$. L-alanine is the amino acid with apolar side-chain –CH3. Based on the research of polar and apolar amino acids, it is well understand the effect of hydroxyl and alkyl group on the interactions of protein's interiors. In this paper, the dilution enthalpies of glycine, L-alanine and l-serine in aqueous potassium chloride solutions of various molalities at 298.15 K and the homogeneous enthalpic interaction coefficients of glycine, L-alanine and L-serine have been reported. The results are interpreted from the point of view of electrostatic interaction and structural interaction.

2. Experimental

2.1. Reagents

Biochemical reagent grade glycine, L-alanine and L-serine were used after recrystallization from methanol–water mixtures and drying in vacuum over P_2O_5 at room temperature for at least 72 h. Analytical reagent grade KCl were recrystallized from distilled water and dried under reduced

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pressure for 72 h at 353 K. The water used for the preparation of solutions was deionized and distilled using a quartz sub-boiling purifier.

Both the aqueous salt solutions, which were used as mixed solvents (water $+$ potassium chloride), and the amino acid solutions (amino acid + potassium chloride + water) were prepared by mass using a Hancpinc FA 1004 balance precise to ± 0.1 mg. All the solutions were degassed and used within 12 h after preparation.

2.2. Calorimetric procedure

The enthalpies of dilution for amino acids in aqueous salt solutions were measured with LKB-2277 BioActivity Monitor at 298.15 K. The solutions were pumped through the mixing-flow vessel of the calorimeter in different rates using a LKB-L010011 microperpex peristaltic pump. The variation in flow rates was less than 0.2%. The flow rates were determined by weighing the masses of the liquids through each tube within 3 min. The variation in flow rates was less than 0.1% both before and after a complete dilution experiment. The liquids passing through tubes A and B were changed in the following sequence:

- 1. A (aqueous salt solution) + B (aqueous salt solution) $$ baseline determined;
- 2. A (aqueous salt solution) + B (amino acid solution) $$ dilution thermal power determined;
- 3. A (aqueous salt solution) + B (aqueous salt solution) $$ baseline re-established.

The enthalpies of dilution $\Delta_{\text{dil}}H_m$ were calculated from the equation:

$$
\Delta_{\text{dil}} H_m = \frac{P(1 + m_1 M)}{m_1 f_2} \tag{1}
$$

in which *P* is the dilution thermal power (μW) , *M* the molar mass of amino acid (kg mol−1) and *f*² the flow rate of amino acid solution (mg s⁻¹). The final molality m_f was calculated from the equation:

$$
m_{\rm f} = \frac{m_{\rm i} f_2}{[f_1(m_{\rm i}M_2 + 1) + f_2]}
$$
 (2)

in which f_1 is the flow rate of diluent (aqueous salt solution).

3. Results and discussion

According to the McMillan–Mayer theory [18], all the thermodynamic properties of multi-components solutions can be expressed by using a virial expansion in *m* which relates the non-ideal contributions of any total thermodynamic function to a series of interaction [param](#page-6-0)eters. If aqueous potassium chloride solution is regarded as "solvent", the excess enthalpy per kg of solvent (H^E) of a solution containing a single amino acid at molality *m* is given by:

$$
H^{E} = h_{2}m^{2} + h_{3}m^{3} + h_{4}m^{4} + \cdots
$$
 (3)

in which h_2 , h_3 , h_4 , etc. are the enthalpic coefficients representing pairwise and, at least notionally, triplet, quarter and higher order interactions between solvated solute species. The molar enthalpy change ($\Delta_{di}H_m$) on diluting a solution of non-electrolytic solute from an initial molality (m_i) to a final molality (m_f) can be written as

$$
\Delta_{\text{dil}} H_m = H_m^{\text{E}}(m_f) - H_m^{\text{E}}(m_i)
$$

= $h_2(m_f - m_i) + h_3(m_f^2 - m_i^2)$
+ $h_4(m_f^3 - m_i^3) + \cdots$ (4)

where $H_m^E(m_i)$ and $H_m^E(m_f)$ are the molar excess enthalpies of the solute in the solutions before and after dilution.

Tables 1–3 give the experimental values $(\Delta_{\text{dil}}H_m)$, together with the initial and final molalities $(m_i$ and $m_f)$ of glycine, l-alanine and l-serine in various aqueous potassium chloride solutions. Tables 4–6 list the coefficients of Eq. (4) [that](#page-2-0) were obtained from least-squares analysis of above results. Since it is difficult to interpret the higher *h* coefficients, only the enthalpic pairwise coefficient h_2 is discussed here. The h_2 [values of gl](#page-5-0)ycine, L-alanine and L-serine in pure water are −466.20, 205.36 and −740.30 J kg mol⁻², respectively, which are in good agreement with those obtained by other workers [23,24]. From Fig. 1, it can be seen that the enthalpic pair interaction coefficients of glycine and L-serine are all negative and become less negative with increasing KCl concentrations, at the same time the $h₂$ coefficients for l-se[rine are m](#page-6-0)ore negative than that for glycine, while pairwise enthalpic interaction coefficients h_2 of L-alanine is positive on which the influence of potassium chloride is not obvious.

It is generally accepted that the h_2 coefficients are attributable to the interaction between two solvated solute

Fig. 1. Variations in enthalpic pair interaction coefficients (*h*₂) of glycine, L -alanine and L -serine with the molality (m) of potassium chloride in aqueous potassium chloride solutions at 298.15 K: (\bullet) glycine; (\blacksquare) L-serine; (\triangle) L-alanine.

molecules and very sensitive to solvent variation. The interaction of non-electrolyte with electrolyte consists of electrostatic and structural interactions. Lilley et al. [25] considered the interactions between electrolyte and amino acids consist of three effects: (a) electrostatic interaction; (b) partial desolvation of solutes; (c) solvent reorganization [19]. The latter two effects belong to the cla[ss of s](#page-6-0)tructural interactions. Desonyers and co-workers [26] gave a general discussion for structural interactions, and thought that in most cases the net effect of the co-sphere overlap on the hydration structure is destructive. Structural interactions make quite a large contribution to the enthalpic function, and sometimes surpasses the effect of electrostatic interaction even predominate [25,27,28]. It can be concluded that the interactions of the ions of electrolyte with the polar groups of non-electrolyte are electrostatic, accompanied by partial

desolvation of the solutes, whereas the interactions with the apolar groups are mainly structural.

Gallardo et al. [29] have measured the homogeneous enthalpic pairwise parameters of some amino acids in water and given two modules of the association for the α -amino acids. One is in a side-by-side manner and the other in a head-o[n-fash](#page-6-0)ion. For zwitterionic amino acids in solution, the configuration likely to contribute most to the pairwise interaction is that in which the molecular association is side-on with the positively charged amino group and its associated co-sphere of one amino acid interacting with the solvated negatively charged carboxyl group of the second molecule [30]. Such strong electrostatic interactions are expected to result in a coalescence of the solvation shells of the charged groups, with a subsequent relaxation of solvent molecules to the bulk solvent. If this effect dominates the pairwise inter-

action, a negative value of *h*² should be observed. Furthermore, the reorganization of solvent due to the coalescence of solvation spheres of the interacting charged groups is also expected to make a negative contribution to h_2 . These effects play an dominant role in the pairwise interaction of glycine as it has no "side-chain-on" carbon. The structural interaction, arising from the apolar side group on the α -carbon, will make positive contribution to h_2 . The exact contribution will depend on the nature of the side-groups involved. Since the apolar part of glycine is shortest, the structural interaction between a pair of glycine molecules is relatively weak. Hence, the values of h_2 for glycine in water are negative (Fig. 1).

In the pairwise association of L-alanine and L-serine, interactions involving the side-chains will be significant. For L -alanine with apolar side-chain $-CH_3$, positive con-

Table 5

Enthalpic interaction coefficients of l-serine in aqueous potassium chloride solutions

m (KCl) (mol kg ⁻¹)	h_2 (J kg mol ⁻²)	h_3 (J kg ² mol ⁻³)	h_4 (J kg ³ mol ⁻⁴)	m_i (mol kg ⁻¹)	
$\overline{0}$	-709.19	194.12	-52.91	$0 - 0.40$	0.9999
0.2149	-652.53	201.67	-65.93	$0 - 0.40$	0.9998
0.4040	-596.64	113.9	22.06	$0 - 0.40$	0.9999
0.4957	-574.69	119.81	28.74	$0 - 0.40$	0.9993
0.7045	-520.88	109.76	3.32	$0 - 0.40$	0.9998
0.8524	-433.49	-60.63	189.61	$0 - 0.40$	0.9998
1.0220	-393.94	-101.74	246.93	$0 - 0.40$	0.9998

tributions to h_2 arise from both the interactions between hydrated hydrocarbon chains [30] and those between the hydrated side-chains and the ionic groups [31]. For L-serine with side-chain functional group –OH that can participate in hydrogen bonding, the contributions to h_2 stemming from hydrox[yl grou](#page-6-0)p–hydroxyl group and hydroxyl group–zwitterionic head group [intera](#page-6-0)ctions should be negative [32,33]. The fact that the value of h_2 for L-serine is more negative than that for glycine supports this view.

In the ternary solutions under investigation (amino acid $+$ potassium chloride $+$ water), the cation and the anion of [elec](#page-6-0)trolyte will undergo electrostatic interactions with the $COO⁻$ and NH₃⁺ groups of amino acids, respectively, and make a negative contribution to h_2 . As is known, in aqueous solutions, hydration sheaths with defined water molecule order are formed around dissolved molecules or ions. The direct interaction provokes partial dehydration of the ions hydration shells, this being an endothermic process and also another endothermic effect resulting from the removal of a number of water molecules from the hydration shell of the polar head of the amino acid [11]. Therefore the partial dehydration of solutes gives positive contribution to h_2 . The

larger concentration of KCl, the more the structure of water will be disrupted, the more positive the contribution from desolvation will be. In conclusion, the pairwise enthalpic interaction coefficients h_2 of glycine and L-serine become less negative with increasing of the molalities of potassium chloride.

The positive values of h_2 of L-alanine suggest that interactions involving alkyl side-chains dominate over the zwitterion–zwitterion interactions. The partial dehydration of solutes gives positive contribution to h_2 of L-alanine in aqueous potassium chloride solutions. The domination of interactions involving alkyl groups changes little with increasing of the molalities of potassium chloride, so the influence of potassium chloride on h_2 of L-alanine is not obvious.

For the interaction between electrolyte and zwitterionic ion, Kirkwood [34,35] proposed an electrostatic theory to estimate the contribution of electrostatic to the pairwise interaction parameters. A feature of the Kirkwood approach is the dominant role played by the dipole moment of the amino acid [in determ](#page-6-0)ining the pairwise interaction parameters. For l-serine with side-chain functional group –OH, the dipole

Table 6

Enthalpic interaction coeffic[ients](#page-6-0) [o](#page-6-0)f l-alanine in aqueous potassium chloride solutions

moment is larger than that of glycine, the electrostatic interaction of l-serine is stronger than that of glycine. In addition, hydroxyl group of l-serine can participate in hydrogen bonding in aqueous potassium chloride solutions. The above contributions to h_2 should be negative, so the h_2 coefficients for l-serine are more negative than that for glycine.

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