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Excess enthalpies of *N*-acetylglycineamide and *N*-acetyl-L-leucineamide in concentrated aqueous solutions of tetramethylurea [1]

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Abstract

A comparison has been made among the values of the enthalpic pairwise interaction coefficients of two *N*-acetylamides of the amino acids glycine (NAGA) and leucine (NALA) in concentrated aqueous solutions of tetramethylurea (TMU) and urea, in pure water, and in pure liquid amides. The second virial coefficients of the excess enthalpies are found to be negative for both the model peptidic molecules in 4 M TMU, as in liquid amides, dimethylformamide (DMF) and fused *N*-methylacetamide (NMA), substances assumed to mimic the core of globular proteins. This is the reverse of what was found for concentrated aqueous solutions of urea (U), where all the enthalpic second virial coefficients were positive. This suggests a completely different mechanism for the denaturation of protein in the presence of urea and TMU, due to different protein–denaturant interactions.

INTRODUCTION

Highly concentrated aqueous solutions of tetramethylurea (TMU) are known as denaturing media for proteins [1–5]. A comparison between the model peptide–model peptide *inter*molecular interactions in this hydroorganic mixed solvent and those for the same species in water, in solvents such as concentrated aqueous solutions of urea [6–8] or guanidinium salts [9], and pure liquid amides, gives information on the changes in intensity of the effects responsible for the delicate balance of the contrasting forces that determine the physiologically stable conformations. In particular, liquid dimethylformamide (DMF) [10, 11] or molten *N*-methylacetamide (NMA) [12] are assumed to mimic the core of globular proteins. In the denaturation process, the *intra*molecular interactions between pairs of folded segments

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of peptidic chains are substituted by *inter*molecular interactions with the aqueous solvent or organic denaturing cosolvent.

With respect to other prevailingly hydrophobic cosolvents, in particular alcohols, TMU offers the advantages of a high boiling point and of being completely water-miscible. A 4 M concentration was chosen as sufficient for promoting polypeptide denaturation at room temperature. It is remarkable that at this concentration the volume fraction of TMU is 0.46 against 0.54 of water.

In this contribution, the excess enthalpies at 298.15 K of aqueous solutions containing 4 M TMU and a diluted *N*-acetylamino acid amide are reported. The species studied were *N*-acetylglycine amide, CH_3CONH-CH_2CONH_2 (NAGA), and *N*-acetyl-L-leucine amide, $CH_3CONHCH$-$[CH_2CH(CH_3)_2]CONH_2$ (NALA).

THERMODYNAMICS

Before discussing the results, the details of the McMillan–Mayer theory of solutions, as adapted by Kauzmann, Friedman and others to non-electrolyte solutions [13–19] will be discussed. This is necessary for a better understanding of the molecular meaning of the physico-chemical quantities used here. For a solution in a mixed solvent of given composition, the excess enthalpy can be defined in the molality scale in the same manner as for an actual binary solution

$$H^E = H - H^{\ominus}_{w,TMU} - m\bar{H}^{\ominus}_x \tag{1}$$

where H is the total enthalpy of the solution (based on 1 kg of constant-composition mixed solvent), H^E is the excess enthalpy of the solution and m the molality of the solute (both on the same basis as H); $H^{\ominus}_{w,TMU}$ is the standard enthalpy of 1 kg of mixed solvent at the chosen fixed composition, and $\bar{H}^{\ominus}_x$ is the partial molar enthalpy of the solute at infinite dilution in this solvent. H^E can be expressed as a power expansion series

$$H^E = h_{xx}m^2 + h_{xxx}m^2 + \ldots \tag{2}$$

where the coefficients are usually obtained from the fitting of enthalpies of dilution according to the following or similar relationships

$$\Delta_{dil}H_m = -(dQ/dt)/P_w = h_{xx}m(m - m') + h_{xxx}m(m^2 - m'^2) + \ldots \tag{3}$$

In eqn. (3), the quantity $-dQ/dt$ is the heat produced per unit time (watts), P_w is the mass flow rate ($kg\,s^{-1}$) of the mixed solvent, and m' is the initial molality (on the basis of 1 kg of the mixed solvent). In this manner, the ternary solution is treated as a binary one containing a single solute species in a unique solvent. The h coefficients are the enthalpic analogues of the excess Gibbs energy virial coefficients g

$$g_{xx} = h_{xx} - Ts_{xx} \tag{4}$$

where s_{xx} is the second virial coefficients of the excess entropy. The h_{xx} coefficients are also related to the excess internal energy coefficients of the solution u_{xx}. The quantity u_{xx} has a compact statistical mechanical expression

$$u_{xx} = \int_0^\infty \frac{\delta[W(r, \Phi_i)/kT]}{\delta[1/kT]} g(r, \Phi_i) 4\pi r^2 \, dr \tag{5}$$

which shows the dependence of this coefficient (through the integration over the entire volume of the solution) on the solute–solute potential of average force $W(r, \Phi_i)$ and the pairwise correlation function $g(r, \Phi_i)$ which are functions of the distance r and of the set of angles (Φ_i) defining the reciprocal orientation of pairs of solutes molecules. However, both $W(r, \Phi_i)$ and $g(r, \Phi_i)$ also depend on the orientations of all the water and cosolvent molecules involved. Consequently, the values of the h_{xx} coefficients depend not only on all the direct solute–solute interactions but also on the solute–solvent interactions, or, rather on the changes in these interactions from extremely dilute solutions up to the actual peptide concentrations considered. In other words, the h_{xx} coefficients depend, in mixed solvents, on the preferential solvation of the components of the mixture for the given solute, and on changes in these solvations on changing the solute concentrations.

EXPERIMENTAL

The enthalpies of dilution of peptide solutions containing a constant ratio of water and TMU were determined at 298.15 K by means of an LKB 10700-1 flow microcalorimeter, according to a procedure reported in previous papers [19–21]. A pair of peristaltic pumps continuously feeds the flow mixing cell of the microcalorimeter, with both the ternary and binary solutions used as the mixed solvent. Special care was applied in all the measurements to ensure the constancy of TMU concentration, which was checked continuously by means of density determinations. A Paar instrument was used for this purpose. Moreover, the same stock of mixed solvent was employed for both preparing and diluting each set of solutions. This is necessary for good reproducibility of the data and the correct use of eqn. (3). The error in the concentration of TMU between the sets of measurements does not introduce any appreciable source of error.

The TMU used was a Fluka product (purum; >99%) and its purity was checked from its physico-chemical properties (density and refractive index). The two *N*-acetylamides, NAGA and NALA (Bachem products), were crystallized from ethanol–ether mixtures and dried in vacuo over P_2O_5. The purity was checked by its DSC melting profile and enthalpy of fusion. Ternary solutions were freshly prepared before each set of dilutions, by dissolving weighed amounts of each amino acid derivative directly in

TABLE 1

Enthalpies of dilution of *N*-acetylglycineamide in aqueous solutions of 4 M tetramethylurea at 298.15 K

Initial molality m_i	Final molality m_f	$\Delta_{dil}H$/J mol^{-1}
0.3964	0.1409	43.0
0.3280	0.1172	35.4
0.3247	0.1162	29.8
0.2930	0.1050	32.3
0.2574	0.0937	27.9
0.2271	0.0817	24.1
0.1911	0.0695	16.4
0.1615	0.0587	18.6
0.1280	0.0465	12.2
0.0972	0.0354	13.4
0.0640	0.0234	8.1

$\sigma = 2.3$.

aqueous 4 M TMU. Bisdistilled, deionized water was employed for all stocks of mixed solvent. Finally, all the solutions and the mixed solvent were filtered on a Millipore membrane to prevent bacterial contamination.

RESULTS AND DISCUSSION

The experimental enthalpies of dilution of the two *N*-acetylamides in aqueous solutions of 4 M TMU are reported in Tables 1 and 2, with the initial and final molalities, calculated on the basis of 1 kg of solvent mixture.

The coefficients h_{xx} for NAGA and NALA in 4 M TMU are listed in Table 3 and compared with the values obtained in pure DMF [10, 11], in molten NMA (at 302 K) [12], in pure water [20–22], and in aqueous 7 M urea [6]. Also reported are the results for *N*-acetyl-L-alanineamide (NAAA) and *N*-acetyl-L-valineamide (NAVA) in the four quoted solvents, for comparison. No h_{xxx} coefficients were determined in the concentration range explored. They are smaller than the respective 95% confidence coefficients so that eqn. (3) was truncated at the first term on the right side.

The most interesting results is the different behaviours of the studied peptides in concentrated solutions of urea and TMU, respectively. In fact for NAGA, the more hydrophilic solute, h_{xx} is negative and only slightly lower in absolute value than that in water; whereas in 7 M urea, it is positive. Moreover, for NALA, h_{xx} is negative in 4 M TMU, in contrast with the large positive values found in water and 7 M urea. These results suggest that aqueous 4 M TMU is a solvent that is very similar to pure liquid DMF and NMA. Negative values of h_{xx} are characteristic of solute–solute

TABLE 2

Enthalpies of dilution of *N*-acetyl-L-leucineamide in aqueous solution of 4 M tetramethylurea at 298.15 K

Initial molality m_i	Final molality m_f	$\Delta_{dil}H$/J mol^{-1}
0.2678	0.1331	26.8
0.2500	0.0961	24.7
0.2499	0.1212	25.5
0.2411	0.0923	21.5
0.2403	0.1170	23.3
0.2287	0.1112	21.8
0.2188	0.0846	18.8
0.2147	0.1046	17.8
0.2064	0.0801	17.8
0.2028	0.0991	17.8
0.1880	0.0918	14.6
0.1766	0.0865	16.2
0.1764	0.0671	15.6
0.1639	0.0804	15.9
0.1563	0.0596	14.0
0.1513	0.0743	12.3
0.1393	0.0685	11.1
0.1054	0.0521	11.5
0.0877	0.0434	9.2

$\sigma = 2.5$

interactions favoured by prevailingly *hydrophilic* interactions that are solvent mediated. These are still operating for NAGA (one peptidic and one amidic group against one methylic and one methylenic group) in water, aqueous TMU, pure NMA and DMF, whereas in 7 M aqueous urea they are screened by the preferential urea–peptide solvation. For NAAA, NAVA and NALA in water and in 7 M urea, and of NAGA in 7 M urea, the positive h_{xx} values are determined by the overwhelming of the hydrophobic and mixed interactions.

TABLE 3

Second enthalpic virial coefficients h_{xx} (J kg mol^{-2}) of *N*-acetylamino acid amides in some solvents at 298.15 K. The 95% confidence limits are reported in parentheses

	DMF [a]	NMA [b]	4 M TMU	W [c]	7 M U [d]
NAGA	−609 (7)	−100 (12)	−164 (9)	−220 (9)	290 (22)
NAAA	−886 (6)	−186 (15)		273 (5)	624 (10)
NAVA	−1432 (50)	−543 (30)		1259 (44)	969 (12)
NALA	−1149 (11)	−415 (23)	−167 (10)	1969 (28)	1430 (21)

[a] Refs. 10 and 11. [b] Ref. 12, measured at 302 K. [c] Refs. 20–22. [d] Ref. 6.

In aqueous 4 M TMU, the solute–solute hydrophobic interactions are masked by the more probable solute-cosolvent hydrophobic interactions. This is particularly evident for NALA, for which h_{xx} becomes small and negative; this is a clear indication of the residual permanence of the hydrophilic interactions alone.

In conclusion, the denaturation mechanism of TMU against proteins is completely different from that of urea. TMU operates essentially by dissolving in a protein globule, swelling it and substituting the *intra*molecular interactions among the apolar side chains of the amino acid residues with the *inter*molecular interactions among the side chains and its own methylic groups. With a high concentration of TMU, the hydrophobic interactions between this cosolvent and the alkylic side chains become so probable that they overwhelm the stabilizing hydrophilic peptide–peptide interactions. In the case of urea, in contrast, direct interactions between the peptidic group and urea are dominant.

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