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THERMODYNAMIC BEHAVIOUR OF SOME UNCHARGED ORGANIC MOLECULES IN CONCENTRATED AQUEOUS UREA SOLUTIONS AND OTHER POLAR SOLVENTS *

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ABSTRACT

A comparison has been made between the values of the enthalpic pairwise interaction coefficients of several organic molecules (peptides, amides and alcohols) in water, in concentrated aqueous solutions of urea, in *N*,*N*-dimethylformamide and in liquid *N*-methylacetamide. The second virial coefficients of the excess enthalpies are found to be positive for all the systems studied in water–urea mixtures. A preliminary analysis, carried out using the Savage and Wood group additivity approach, suggests that, in concentrated aqueous solutions of urea, this arises from the peptide–peptide or hydroxyl–hydroxyl, and the apolar–apolar group contributions, all being positive, and these overwhelm the negative contributions from the polar–apolar group interactions. A remarkable feature is the inversion of the signs of the peptide–peptide, hydroxyl–hydroxyl, peptide–methylene and hydroxyl–methylene, group interactions when compared with those which prevail in pure water. This suggests a completely different solvation and interaction mechanism in concentrated urea solutions. The enthalpic contributions from apolar–apolar group interactions in the mixed solvent, in turn, are higher than the values found in water.

Some comments are made on the behaviour of some of the above solutes, in the liquid amide solvents *N*,*N*-dimethylformamide and *N*-methylacetamide.

INTRODUCTION

It is well known that the unique biologically active conformations adopted by proteins and other naturally occurring macromolecules result from delicate balances of different, and frequently opposing, intramolecular and intermolecular interactions. Concentrated aqueous solutions of urea are often used as a denaturing medium for biopolymers and the high concentra-

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tions required by this conformational perturbant, for it to be effective, indicate that urea is a non-specific agent, which operates with different mechanisms on the various elements of macromolecular structures. It seems probable that there is, for example, competitive solvation for the polar groups on the surface of globular proteins, between the water and urea molecules, and also competitive intermolecular interactions of urea with peptide groups which can disrupt the intramolecular peptide–peptide hydrogen-bonding interactions. It also seems likely that there is some significant perturbation of the hydrophobic associative interactions which are present in the proteins. Often in the literature, the increased solubility of lighter hydrocarbons, in concentrated aqueous urea, with respect to their solubility in water, has been inferred to be a proof of the lack of apolar group–apolar group interactions in these mixtures. However, this is not the only possible rationalisation and, for example, one of the consequences of the model of the hydrophobic interactions proposed by Ben-Naim, which has been applied to the solubility of methane and ethane in water-urea mixtures, is that apolar–apolar interactions are reinforced by the presence of urea [1]. To investigate the changes undergone by both the apolar group and polar group interactions in water–urea solutions, we are currently carrying out a joint research programme, in our laboratories, on the excess thermodynamic properties of organic solutes such as amides [2], *N*-acetylamides of amino acids and peptides [3], alkanols (both monofunctional [4] and bifunctional [5]) and sugars [6].

It was suggested, a while ago, that comparisons between the interactive properties of solutes in water and in liquid amides, might well give some indication of the forces that regulate the folding of polypeptides during and after their biosynthesis, whereas comparisons between the same properties in water and in urea–water mixtures would be useful in giving insight into the factors that promote the unfolding of the biopolymers [7] in such solutions.

THERMODYNAMICS

The relatively large amount of calorimetric data currently at our disposal concerning the enthalpies of dilution of substances related to molecules of biological interest, allows us to suggest a preliminary rationalisation of the pairwise interaction coefficients in different media. According to the McMillan–Mayer theory of solutions [8], as adapted by Kauzmann, Friedman and co-workers to nonelectrolyte solutions [8–10], the excess enthalpy can be defined, using the molality scale to express solution composition, as

$$H = H^{E} - H_1^0 - mH_x^0 \qquad (1)$$

If one considers solutions containing only one solute, then the excess

enthalpy can be expressed as a power series in the molality (m) of the solute:

$$H^{E} = h_{xx}m^2 + h_{xxx}m^3 + \ldots \tag{2}$$

In these two equations, H is the total enthalpy of the solution containing 1 kg of solvent (either pure or mixed), H^{E} is the excess enthalpy of the solution; H_1^0 is the standard enthalpy of 1 kg of pure or mixed solvent and H_x^0 the standard state (infinite dilution) partial molar enthalpy of the solute. The homotactic enthalpic interaction coefficients (h_{xx}, h_{xxx}, etc.) of eqn. (2), are usually obtained from fitting of enthalpies of dilution, according to the following, or similar, relationships.

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

where m' and m are respectively the initial and final molalities of the solute and $\Delta_{dil}H_m$ is the molar enthalpy of dilution. The coefficients h_{xx}, etc. are the enthalpic analogues of the excess free energy virial coefficients g_{xx} and are related to these by

$$h_{xx} = \left[\partial(g_{xx}/T)/\partial(1/T)\right]_P \tag{4}$$

$$g_{xx} = h_{xx} - Ts_{xx} \tag{5}$$

$$h_{xx} = u_{xx} + \alpha RT^2\left[g_{xx} - (RTM_1/2000) - \left(\Phi_2^{0E}/\alpha V_1^0\right)\right] \tag{6}$$

In these, s_{xx} and u_{xx} are the second virial coefficients of excess entropy and excess internal energy of the system considered, α is the thermal expansion coefficient of the solvent, Φ_2^{0E}, is the standard state partial molal expansibility of the solute and M_1 and V_1^0 are, respectively, the molar mass and the molar volume of the solvent. All these interactions can be related to molecular events in a formally correct statistical mechanical way. For example, the quantity u_{xx} can be expressed as

$$u_{xx} = \int_0^{\infty} \frac{\partial\left[W(r,\Phi_i)kT\right]}{\partial\left[1/kT\right]} g(r,\Phi_i)4\pi r^2\,dr \tag{7}$$

This shows that this coefficient is directly related to the solute–solute potential of average force $W(r,\Phi_i)$ and to the pairwise correlation function $g(r,\Phi_i)$. It should be noted that the integration is performed over the entire volume of the solution and that the coefficient depends on both the distance (r) separating the molecules and on the sets of angles (Φ_i) defining the reciprocal orientation of pairs of solute molecules. However, these quantities depend also on the orientations of all the water molecules involved and consequently the values of the h_{xx} coefficients depend, not only on the direct solute–solute interactions, but also have contributions arising from the solute–solvent interactions.

When making comparisons between data obtained in different solvents and in solvent mixtures, it would be preferable to convert the direct

calorimetric h_{xx} coefficients to u_{xx} coefficients, since these are more closely related to molecular events. However, to perform transpositions of this type one needs values of all the properties shown in the second term on the right hand side of eqn. (6), but usually, and certainly for the systems considered here, such information is not available. In this paper, when dealing with different solvents and solvent mixtures, we have used the molality scale and so must recognise that uncertainties can be introduced by doing this. In some ways it would be preferable to compare the results obtained in different solvent systems on a molar scale, but given that in most of the interpolation we address sign changes in interaction coefficients, this has not been done. However, in some of the following, we will compare the h_{xx} values taking into account, where necessary, the different densities of the pure or mixed solvents.

RESULTS AND DISCUSSION

The information that we wish to discuss in this paper is collected in Table 1. The first group of solutes comprises some simple amides, formamide (FA), acetamide (AA), and propionamide (PA), their *N*-methyl derivatives (NMF, NMA, NMP), their *N*,*N*-dimethyl derivatives (DMF, DMA, DMP), and some *N*,*N*-diethyl derivatives (DEF, DEA). These amides have been studied in aqueous 8 M urea solutions [2], in pure water [11–15], in DMF [16,17] at 298 K and in liquid NMA at 305 K [7,18]. Other amides have been studied in DMF only [19] and are not considered here. The second group of solutes consists of the *N*-acetyl amides of the following amino acids: glycine (NAGA), L-alanine (NAAA), L-valine (NAVA), L-leucine (NALA), L-isoleucine (NAIA), L-proline (NAPA) and L-phenylalanine (NAFA). The measurements have been carried out in aqueous 7 M urea [3], in pure water [20–24] and in DMF [25] at 298 K and in liquid NMA at 305 K [7,18]. The measurements on the third group of solutes (alkanols) have been performed at 298 K in aqueous 7 M urea only [4], because of the large number of data reported in the literature for their aqueous dilute solutions and because they are of interest essentially for testing the conclusions reached on the qualitative changes in the group interactions of solutes between water and concentrated urea solutions [4,14,26–29]. Complementary data are reported for bifunctional alcohols in a companion paper [5].

There are several points to be made about the results which have been obtained. As was mentioned above, most of our attention will be directed towards the results obtained, for amidic and peptidic species, in concentrated urea solutions, and in particular comparing these to the results obtained in pure water. Some comments will however be made about the available information on amides in non-aqueous solvents and on alcohols in urea–water mixtures.

TABLE 1

Enthalpic second virial coefficients (J kg mol^{-2}) for uncharged peptides, amides and alkanols in water and polar solvents and mixtures at 298.15 K (NMA at 305 K)

Solute	Solvent [a]			
	W/U8M	W	DMF	NMA
FA	32 (4) [b]	−115 (2) [c]	119 [h]	96 (3) [j]
AA	157 (5) [b]	1 (14) [d]	−350 (3) [i]	30 (1) [j]
PA		249 (21) [e]		
NMF	352 (3) [b]	272 (2) [f]	4 [h]	−10 (1) [j]
NMA	538 (24) [b]	236 (11) [f]	−124 (1) [i]	(0)
NMP		636 (24) [f]		
NBA		1477 (24) [f]		
DMF	810 (22) [b]	737 (7) [c]	(0)	−70 (3) [j]
DMA	1060 (47) [b]	1081 (28) [g]	4 (1) [i]	94 (9) [j]
DMP		1797 (9) [g]		
DEF		1767 (19) [d]		
DEA		2355 (30) [d]	−11 (1) [i]	
	W/U7M			
NAGA	290 (22) [k]	−220 (9) [l]	−609 (7) [q]	−100 (12) [j]
NAAA	624 (10) [k]	273 (5) [m]	−886 (6) [q]	−186 (15) [j]
NAVA	1101 (36) [k]	1259 (44) [l]	−1432 (50) [q]	−543 (30) [j]
NALA	1433 (29) [k]	1969 (28) [n]	−1149 (11) [q]	−415 (23) [j]
NAIA	2300	2000		−821 (30) [j]
NAPA	892 (16) [k]	660 (28) [o]		−80 (9) [j]
NAFA		1049 (53) [p]	−982 (20) [q]	−1045 (265) [j]
MeOH	167 (6) [r]	224 (3) [s]		
EtOH	238 (6) [r]	243 (10) [t]		
iPrOH	278 (6) [r]	339 [f,u]		
nPrOH	513 (6) [r]	559 (14) [t]		
iBuOH	743 (41) [r]	1000 [f,v]		
sBuOH	874 (7) [r]	916 [f,v]		
tBuOH	639 (81) [r]	656 (33) [t]		
nBuOH	846 (24) [r]	1245 (11) [s]		
nPeOH	1190 (25) [r]	1724 (25) [r]		

[a] W/U8M, 8 M aqueous urea; W/U7M, 7 M aqueous urea; W, water; DMF, *N*,*N*-dimethylformamide; NMA, *N*-methylacetamide. The number in parentheses represents the 95% confidence limits of the coefficients.

[b] Ref. 2, [c] Ref. 11, [d] Ref. 12, [e] Ref. 13, [f] Ref. 14, [g] Ref. 15, [h] Ref. 16, [i] Ref. 17, [j] Ref. 7, 18, [k] Ref. 3, [l] Ref. 20, [m] Ref. 21, [n] Ref. 22, [o] Ref. 23, [p] Ref. 24, [q] Ref. 25, [r] Ref. 4, [s] Ref. 26, [t] Ref. 27, [u] Ref. 28, [v] Ref. 29.

The most striking feature of the experimental results is that all of the enthalpic virial coefficients are positive, in concentrated urea, for all of the systems studied. This is in contrast with what is found in water, where it is generally found that those solutes, which on balance are hydrophilic, have negative enthalpic virial coefficients.

The data presented in Table 1 show some other significant features. For the more hydrophobic compounds, namely for the peptidoamides NAVA and NALA, usually the coefficients are less positive than those in water. The same feature is observed for the alkanols, with the exception of ethanol and *t*-butanol (for both of these, the values are within the experimental uncertainties). For amides and peptides of medium net hydrophobicity, the coefficients are all positive but more positive than those in water.

In an attempt to rationalise the data, it was decided to use the Savage and Wood additivity of groups (SWAG) approach to molecular interactions [14]. According to this, the enthalpic second virial coefficient is given by the sum of all the contributions obtained by coupling each group i of the solute molecule x with each group j of the solute molecule y (of the same or different species)

$$h_{xy} = \sum n_i^x n_j^y H_{ij} \tag{8}$$

This approach, even though it is recognised that it contains some approximations, is, in part, implicit in the cluster-expansion treatment of solutions [30–32]. It is also of considerable practical interest and importance, since it seems to work tolerably well in those situations where, for a group of structurally similar compounds, the interactions are weak and non-specific. One of the significant, and relatively novel, merits of the SWAG approach is that it recognises the contribution of the cross interactions between polar and apolar groups and the contribution that these make to the net interaction between molecules.

In the analysis of the data, the groups considered were the peptide (amidic) group and, as in earlier work, the CONH and the $CONH_2$ were considered to be equivalent; the OH group; and the CH_2 group. As is now customary, it was assumed that other apolar residues could be treated as methylene groups and when doing the group counting, a methyl group was taken to be equivalent to 1.5 CH_2 groups and a methyne group was taken as 0.5 CH_2 groups. The systems considered in the present paper are the alkanols, peptides and amides, (the information on NAIA should be considered as preliminary and so has not been included in the analysis). The molecules containing the tertiary amide (secondary peptide) group, NAPA, DMF, DMA, were also not included in the data analysis since this would have involved the introduction of another type of group into the additivity scheme, and would in turn have necessitated the introduction of three more group interaction coefficients. In the analysis of the data on alcohols, the information on the homotactic coefficients of some 9 diols [5] was included. As was mentioned above, the experimental h_{xx} values were normalised using densities to approximately correct for differences in the urea concentrations employed.

The results of the SWAG analysis are given in Table 2, where the 95% confidence limits for each coefficient and the standard deviation of the fits

TABLE 2

Group contribution coefficients (J kg mol^{-2}) to the enthalpic second virial coefficients in water and in concentrated aqueous urea solutions at 298.15 K

Group i	Group j	H_{ij}		
		Urea–water	Water	Water
CONH	CONH	141 (56)	−292(9); peptides −260(11); amides	
CONH	CH_2	−51 (21)	80(6)	
CH_2	CH_2	54 (9)	25(3); amides and peptides	38(3); alcohols
OH	OH	147 (59)		−61(5)
OH	CH_2	−58(24)		34(4)
σ		118	136	172
Number of systems		26	80	57

are also reported. Introducing the excluded values (and assuming that tertiary, secondary and primary amide groups are all equivalent) resulted in a marked worsening of the fitting. Table 2 includes, for comparison, the results of the SWAG analyses, applied to aqueous solutions. It should be pointed out that the data fittings, for the concentrated urea solutions, converged more rapidly than for aqueous systems. In other words, the statistical basis for applying the SWAG method successfully seems to need a less numerous data set in concentrated urea than in the aqueous medium. The implications of this are, firstly, that the interactions between solvated groups, in the mixed solvent, are less orientationally specific, and secondly, that the interactions are generally weaker. Direct interactions, such as dipole–dipole interactions, between the unsolvated molecules, would contain important orientationally specific contributions and we feel that these are not indicated by the present results. The dominant contribution to the enthalpy changes associated with the interaction between two groups, is therefore, likely to stem from interactions between the solvent molecules, which are proximate to the groups. Since the local composition of the group solvation regions, and the properties of those solvent molecules, are necessarily determined, to some extent at least, by the nature of the group, it would seem that the SWAG treatment is more applicable in urea–water mixtures, because subtle and perturbing effects such as nearest neighbour interactions are damped (or screened) by the intervening solvent molecules.

It must be stressed that, notwithstanding the fairly good fit to the data shown in Table 2, there are still significant uncertainties associated with the various coefficients which have been obtained. It seems likely that these arise, to a considerable extent, from stereochemical effects which are not taken into account by the group additivity approach, and also from solva-

TABLE 3

Contributions to the net enthalpic virial coefficient of NAVA in J kg mol^{-2}

	Contribution		
	Peptide–peptide	Methylene–methylene	Peptide–methylene
7 M urea solution	564	1634	−1122
Water	−1168	756	1760

tion region overlaps and intramolecular electronic rearrangements, stemming from, for example, inductive effects.

It is instructive to compare the contributions to the net enthalpic virial coefficient, of the peptidic molecule, NAVA. This species has approximately the same coefficient in concentrated urea solutions as in water and, using the group coefficients given in Table 2, one can calculate the data of Table 3. Because of deficiencies in the SWAG treatment, the sums of either of the two rows are not identical with the experimental values, but notwithstanding this, the changes in the two solvent systems are quite remarkable and highlight the very significant changes which are occurring at the molecular level, which are not evident from perusal of the coefficients in isolation.

The most striking result, reported in Table 2, is the change in the sign of the coefficient representing peptide group–peptide group interactions. In water, for the secondary amide (primary peptide) group, this is large and negative, but changes to being relatively large and positive in the concentrated urea solutions. A similar, although less marked, change is observed for the homotactic hydroxyl group coefficient. In other words, for both the amide and the hydroxyl groups, the interactions change from being thermochemically attractive in water, to being thermochemically repulsive, as the solvent medium is changed to concentrated urea solutions. To some extent, this is to be expected, since for example, there is some recent evidence [12] which indicates quite clearly that in dilute aqueous solutions urea interacts in a favourable way with amide and peptide groups, i.e., the urea–peptide group interaction is stronger than the sum of the urea–water interaction and the peptide group–water interaction. The consequence of these is that, in urea solutions, it is to be expected that the polar CONH groups will be preferentially solvated by urea molecules and, at the high concentrations used (the urea occupies at least 60% of the solution volume), the solvation region of the amide groups will be to all intents "saturated" by urea molecules. It also seems apparent that the urea–peptide group interaction is thermochemically more favourable than the peptide group–peptide group interaction, although the discrimination is not very great, and so it would appear entirely reasonable that in urea solutions the solvated amide groups would have no tendency to self-interact. The experimentally deduced posi-

tive coefficient suggests that the orientation of the urea molecules solvating the peptide groups is such that if two such groups approach each other in solution, then repulsion between the solvated entities results.

A second noteworthy feature of the results given in Table 2 is the magnitude of the methylene–methylene group interaction coefficient in the urea–water solvent medium. It has approximately twice the value of the corresponding term in water for peptidic and amidic solutes [11–15,20,31,32] and is rather higher than the value found in water for mono- and polyfunctional alcohols. It seems that one feature, i.e. a positive enthalpic interaction coefficient term, which is generally accepted as being a manifestation of the unusual and characteristic hydrophobic effect of apolar groups in water, is enhanced in the mixed solvent. It is fair to say that at the outset of these experiments on solute interactions in urea–water mixtures, we would not have predicted this rather surprising observation. However, it would seem that this observation is at least consistent with the model proposed by Ben-Naim and Yaacobi [1].

In contrast to the observation made earlier regarding the polar group–urea interactions in water, it has been found for the same systems that there is a thermochemically repulsive interaction between urea and hydrophobic residues in water. Now, given that the apolar residues are constrained to be present in the mixed solvent system, it seem reasonable that they are solvated preferentially by water. It seems likely that this situation arises, not from any significant attraction occurring between apolar residues and water, but rather that the solvation region adopts the energetically most favourable composition and, of those possible, that containing a preponderance of water is the least endothermic. Some support for this comes from the fact that urea is a more polar species than water. It is envisaged that the water molecules in the solvation regions of the apolar groups will tend, to some extent, to interact strongly with proximate water and urea molecules. The consequence of this is that the solvation water will retain some of the gross structural characteristics of water around the apolar groups in dilute aqueous solutions, or at least will be involved in a more stable network of interactions than those which pertain in the bulk mixed solvent. In the absence of other information, we suggest that the qualitative explanation for the rather surprising observation that in urea solutions the solvated apolar group–apolar group interactions exhibit a positive enthalpic coefficient, is analogous to that used for rationalising the interactions between apolar groups in water. It is postulated that the water molecules which surround apolar groups, and which are also interacting with urea molecules more distant from the apolar groups, will be released when apolar residues interact intermolecularly, with a consequent increase in enthalpy arising from the broken water–water hydrogen bonds.

The coefficient representing the interaction between the methylene group and the peptide group, is of different sign in the urea–water mixtures from

that in aqueous systems. In water, the term for CONH–CH_2 interactions, is relatively large and positive, and this is surmised to arise, in large part, from the incompatibility of the solvation regions of the chemically disparate groups. A similar rationalisation can be made for the interaction between the hydroxyl and methylene groups in water. In the urea–water mixtures, however, the polar–apolar group coefficients are negative, which is indicative of thermochemically attractive and favoured interactions. It does not seem conceivable that direct interaction between such chemically different groups would be attractive and so we must conclude that the negative group coefficients arise from interactions between solvating molecules on the peripheries of the solvation regions of the two interacting species. It is difficult to be more precise than this since there is, at the moment, no firm information on the nature and composition of the solvation regions in the mixed solvent considered, but one can speculate that the urea molecules in the solvation shell of the peptide groups are electronically perturbed because of their relatively strong interaction with the peptide groups and as a consequence will interact more strongly with the polar molecules (water and urea) in the solvation shell of the apolar group. A similar argument can be invoked for the OH–CH_2 interaction in this solvent system. In other words, for the polar—apolar interactions we are suggesting that solvent-separated solute pairs have some marginal stability induced by the solvation regions in urea–water mixtures.

The preceding considerations on the peptide–peptide and CH_2–CH_2 interactions are of some relevance to the unfolding of proteins, induced by urea–water mixtures (for structural reasons the peptide–CH_2 contributions are probably relatively unimportant in globular proteins). This will not be pursued here but will be addressed at some length in a future publication.

Even in the absence of free energy and entropy data, the values obtained for the group coefficients strongly suggest that the apolar interactions (albeit rather modified from those of a hydrophobic nature prevailing in water) still seem to operate and make significant contributions to net molecular associative events in concentrated aqueous urea solutions. In spite of the fact that hydrophobic interactions are usually associated with the organisation of a regular, modified "ice-like" structure of water around the periphery of apolar solutes, and even though the chaotropic nature of urea promotes a disruption of the "ice-like" clusters of water, the polar liquid mixture, formed by urea and water in comparable proportions, probably consists of distorted clusters of both components internally connected by a network of transient hydrogen bonds and dipole–dipole interactions [33,34]. If this picture is physically based, then it can be hypothesised that, for example, apolar groups would be excluded from these networks of polar interactions and statistically "pushed" each one against the other. Focussing attention on the solvent rather than on the solute, the term "lipophobic" can be used to define the effect in mixed-solvent and non-aqueous systems.

This aspect brings us to consider, very briefly, solutions of amidic solutes in liquid amides. In general, the h_{xx} coefficients have the opposite sign to those found in water and one sees positive values for some of the more hydrophilic solutes and negative values for the less hydrophilic solutes. This point is important because DMF, and especially liquid NMA, can be considered as liquids that mimic the core of globular proteins [7,18]. Consequently, studies of solutions in these liquids, can give useful information about the peptide–peptide interactions in globular protein interiors. The general trend towards increasingly negative values of the h_{xx} coefficients, with increasing molecular size, of the solutes, are clearly due to the prevailing hydrophilic interactions arising from hydrogen bonds, dipole–dipole and other effects mentioned before. However, the inversion between NAVA and NALA is noticeable, as well as the behaviour of the aromatic peptide NAFA. This can be explained on the basis of preferential solvation and of specificity of solute–solute interactions differently mediated by each of the two particular solvents. Also remarkable are the positive values of the virial coefficients for FA and other small amides. For FA, for example, it seems likely that the value and the sign arises from strong solute–solvent interactions overcoming the opposing solute–solute ones and, in part, from the relatively small dimensions of this solute to those of the solvent.

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