

Interactions in solutions: A calorimetric study of pure and mixed solvent systems

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Abstract - Enthalpies of solution of electrolytes as well as nonelectrolytes in several pure and mixed solvents are discussed. The resulting enthalpies of transfer from one solvent to another in solvent systems containing water show the influence of hydrophobic hydration for solutes containing alkyl groups, *viz.* tetraalkylammonium bromides, alkanols and substituted ureas.

Enthalpies of dilution give information on the energetic aspects of solute-solute interactions. Here too, the mediating solvent is important and when hydrophobic solutes are involved water distinguishes itself from other solvents. Attention is given to a group of dipeptide amides as model compounds for intramolecular interactions of globular proteins. In amidic solvents with functional groups similar to those of the dipeptides, the influence of size and shape of the alkyl groups and that of chirality is discussed.

INTRODUCTION

Although they mutually influence each other, it is convenient to divide molecular interactions in solutions into solute-solvent interactions, or solvation, and solute-solute interactions. The energetic aspects of both types of intermolecular interactions can be investigated directly by different calorimetric techniques. Rather than presenting a survey on the calorimetric methods, this contribution will discuss results of calorimetric investigations on solvation and solute-solute interactions recently performed in the author's laboratory.

With respect to solvation, systematic studies have been carried out in aqueous and non-aqueous solvent systems, pure as well as mixed, of solutes carrying alkyl groups of different size and shape. Electrolytes as well as non-electrolytes will be considered. The interest in the behaviour of alkyl groups was triggered by the peculiar way of solvation of these entities in water, the so-called 'hydrophobic hydration'.

Our studies on solute-solute interactions were focused also on the influence of alkyl groups. In order to avoid long range Coulombic effects only non-electrolytes have been investigated. Again, aqueous and non-aqueous solvents were involved. By using special model compounds, *i.e.* substituted amides and dipeptide amides, it was tried to get information on the factors possibly influencing the spontaneous folding of globular proteins in water.

ENTHALPIES OF SOLUTION IN PURE AND MIXED SOLVENTS

All enthalpies of solution discussed in this contribution have been measured calorimetrically using commercially available instruments. Details about the calorimetric procedure and the purification of solutes and solvents can be found in the original work (ref. 1a,b). Values presented refer to infinite dilution and, unless indicated otherwise, to 25 °C.

Tetraalkylammonium compounds

The peculiar solvation behaviour of tetraalkylammonium salts in water is demonstrated in Fig. 1 by comparing the enthalpies of solution of tetrapropyl- and tetrabutylammonium bromide with those of three simple alkali bromides, KBr, RbBr and CsBr, in water and in two non-aqueous solvents. The latter are *N*-methylformamide (NMF) which is protic and, like water, highly associated in the liquid state, and *N,N*-dimethylformamide (DMF), an aprotic, non-associated liquid. For one solute the differences between the enthalpies of solution in the solvents reflect directly the changes in solvation.

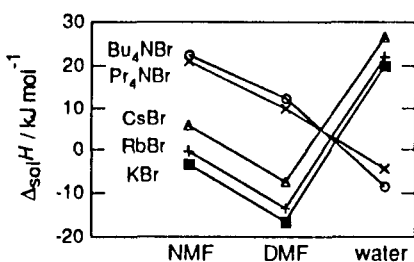


Fig. 1. Enthalpies of solution of several bromides in DMF, NMF and water.

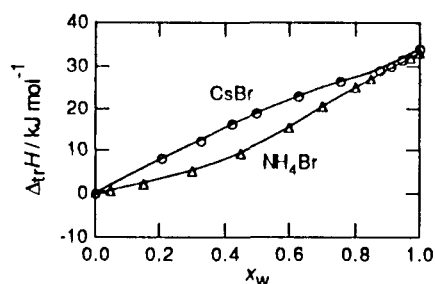


Fig. 2. Enthalpies of transfer of CsBr and NH_4Br from DMF to water.

Figure 1 clearly shows that these differences are of similar magnitude for the transfers between the non-aqueous solvents, but become different for the transfers from NMF and DMF to water. For the alkali bromides the enthalpies of transfer are endothermic and virtually constant, for the organic bromides they are exothermic and dependent on the size of the alkyl groups. This picture is general for transfers involving water and Table 1 gives quantitative results for the enthalpies of transfer of five alkali bromides and four tetraalkylammonium bromides between two aprotic non-aqueous solvents (dimethylsulphoxide, DMSO, and DMF), a protic and an aprotic non-aqueous solvent (NMF and DMF), and a non-aqueous solvent (DMF) and water. The hydrophobic hydration of the tetraalkylammonium ions reveals itself in exothermic contributions to the enthalpies of transfer ranging from about -20 kJ mol^{-1} for Me_4NBr to approximately -50 kJ mol^{-1} for Bu_4NBr .

TABLE 1. Values of $\Delta_{\text{tr}}H / \text{kJ mol}^{-1}$ for transfers from DMF to DMSO, DMF to NMF and DMF to water of alkali and tetraalkylammonium bromides.

	DMF→DMSO	DMF→NMF	DMF→water		DMF→DMSO	DMF→NMF	DMF→water
LiBr	6.0	14.3	28.7	Me_4NBr	-3.6	11.0	8.2
NaBr	6.0	13.5	30.3	Et_4NBr	4.2	11.7	-3.2
KBr	5.0	12.8	36.2	<i>n</i> - Pr_4NBr	6.1	10.9	-14.2
RbBr	5.6	13.6	35.6	<i>n</i> - Bu_4NBr	8.3	10.0	-20.9
CsBr	5.0	13.5	33.8				

The difference in behaviour between alkali bromides and tetraalkylammonium bromides is also present in mixed solvents containing water. Figure 2 shows that the enthalpies of transfer of the non-hydrophobic salts CsBr and NH_4Br from DMF to mixtures of water and DMF, change gradually from one pure solvent to the other.

However, the enthalpies of transfer of Bu_4NBr in the same mixtures show a completely different trend. Upon introduction of water in pure DMF, the enthalpy of transfer of Bu_4NBr changes linearly with the mole fraction of water, x_w , in an endothermic sense up to $x_w \approx 0.7$. At higher mole fractions of water the enthalpies of transfer shift sharply in exothermic direction towards a large, exothermic enthalpy of transfer to pure water (see Fig. 3). In non-aqueous mixtures this shift is absent. As is shown in Fig. 4,

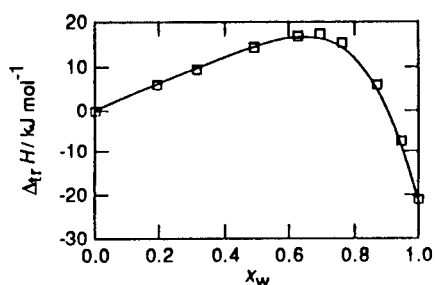


Fig. 3. Enthalpies of transfer of $n\text{-Bu}_4\text{NBr}$ from DMF to mixtures of water and DMF.

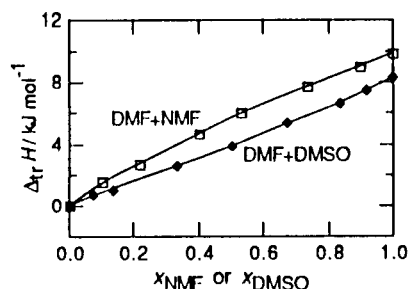


Fig. 4. Enthalpies of transfer of $n\text{-Bu}_4\text{NBr}$ in non-aqueous solvent mixtures.

the enthalpies of transfer of Bu_4NBr in mixtures of DMF and DMSO or DMF and NMF change practically linearly with the solvent composition. A comparison of the enthalpies of transfer of NH_4Br and BuNH_3Br in mixtures of water and DMF demonstrates that the exothermic shift in the aqueous solvent systems is due to the presence of alkyl groups. In Fig. 5, the curves relating the enthalpy of transfer of these salts and the solvent composition are nearly coinciding at low water content. In water-rich solvents, the introduction of a single butyl group affects already the solvation substantially and shifts the enthalpy of transfer in an exothermic sense.

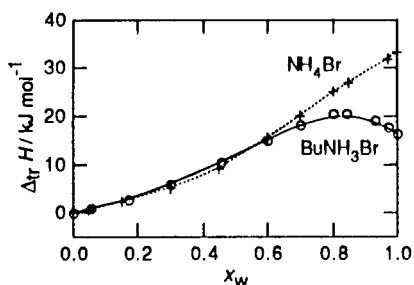


Fig. 5. Enthalpies of transfer of NH_4Br and $n\text{-BuNH}_3\text{Br}$ in mixtures of water and DMF.

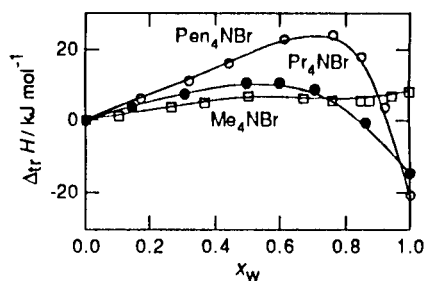


Fig. 6. Enthalpies of transfer in mixtures of water and DMF for $n\text{-Pr}_4\text{NBr}$, $n\text{-Bu}_4\text{NBr}$ and $n\text{-Pen}_4\text{NBr}$.

Figure 6 gives the changes of the enthalpies of solution in mixtures of water and DMF for three tetraalkylammonium bromides as a function of solvent composition. The exothermic shifts occurring at higher x_w are only comparable for Pr_4NBr , Bu_4NBr (see Fig.3) and Pen_4NBr . The magnitude of the shift increases with the size of the alkyl groups and for Bu_4NBr it is approximately four times that for BuNH_3Br presented in Fig. 5. This suggests that at least in water-rich mixtures the alkyl groups are independently solvated and contribute additively to the total changes.

In pure water, the physical chemical properties of tetraalkylammonium salts are largely the result of hydrophobic hydration of the cations (ref. 2). Due to the presence of alkyl groups, the tetraalkylammonium ions are surrounded by a clathrate-like 'cage' of water molecules. If we assume that this type of hydration is rapidly affected by the addition of an increasing amount of cosolvent to water, the behaviour of the tetraalkylammonium bromides in mixtures of water and DMF can be easily described. Several simple model approaches (ref. 3,4) lead to an equation reflecting satisfactorily the experimental results over the complete composition range by means of only two adjustable parameters :

$$\Delta_{\text{sol}} H(M) = (1 - x_w)\Delta_{\text{sol}} H(\text{DMF}) + x_w\Delta_{\text{sol}} H(W) + (x_w^n - x_w)Hb(W) \quad (1)$$

In this equation, $\Delta_{\text{sol}} H(M)$ is the enthalpy of solution of the salt in the solvent mixture, $Hb(W)$ is the enthalpic contribution of hydrophobic hydration in pure water, and n is a parameter reflecting the number of water molecules involved in the hydration of one alkyl group. For Pr_4NBr , Bu_4NBr and Pen_4NBr the equation applies very well. The resulting values of $Hb(W)$ are : $-39.6 \text{ kJ mol}^{-1}$, $-52.8 \text{ kJ mol}^{-1}$, and $-58.0 \text{ kJ mol}^{-1}$, respectively. The mean deviations between experimental and calculated

values are less than 0.4 kJ mol^{-1} . The trend in the obtained values of n is correct. However, their magnitudes are physically unrealistic, but this can be remedied by introducing additional model assumptions (see ref. 1a). In Fig. 6 it is clear that the shape of the curve for Me_4NBr (to a lesser extent also that for Et_4NBr) differs substantially from those of the larger tetraalkylammonium salts. For this salt the relative influence of the charge of the R_4N^+ ion becomes more important and hydrophobic contributions are much less dominant.

One of the features of the model approach is the assumption of independent solvation of the alkyl groups in the solute molecules. This aspect has been investigated by measurements on asymmetric tetraalkylammonium bromides. In Fig. 7 enthalpies of solution in mixtures of water and DMF are presented for Me_4NBr , Me_3BuNBr , MeBu_3NBr and Bu_4NBr . Since the enthalpies of transfer yield overlapping curves, the plot gives the enthalpies of solution. That means that the shapes of the curves are important and not their location. As Fig. 7 shows, the shape of the curves changes gradually from Me_4NBr to Bu_4NBr . A numerical analysis reveals that the curves of the asymmetric compounds can be calculated from the others by applying simple additivity.

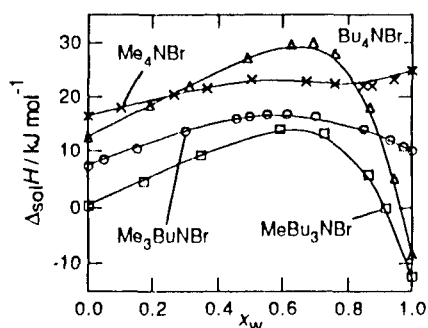


Fig. 7. Enthalpies of solution of Me_4NBr , Me_3BuNBr , MeBu_3NBr and Bu_4NBr in mixtures of water and DMF.

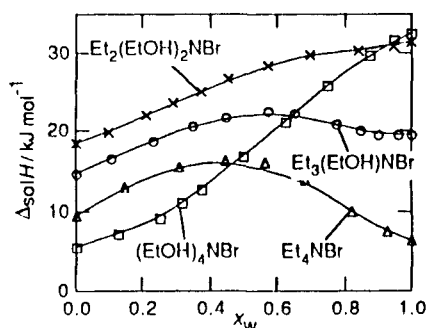


Fig. 8. Enthalpies of solution of several OH substituted tetraalkylammonium bromides in mixtures of water and DMF.

It is possible to affect the nature of the R_4N^+ ion significantly by introducing terminal OH groups in the alkyl groups. The result for the solvation of the solute Et_4NBr is shown in Fig. 8. From the shape of the curves it appears that $\text{Et}_3(\text{EtOH})\text{N}^+$ is already less hydrophobic than Et_4N^+ and that the hydrophobicity decreases as additional terminal OH groups are substituted in the other ethyl groups. The compound with four terminal OH groups, $(\text{EtOH})_4\text{NBr}$, is not hydrophobic at all and its curve in Fig. 8 resembles that of CsBr (see Fig. 2). Again, additivity occurs. The exothermic shift at high water content in the curve for $\text{Et}_3(\text{EtOH})\text{NBr}$ is approximately 75% of that in the curve for Et_4NBr .

Since hydrophobic hydration is related to the structure of liquid water, it is influenced by temperature. This is reflected by the enthalpies of solution of the hydrophobic solute Bu_4NBr in mixtures of water and DMF. Figure 9 gives the enthalpies of solution at 5, 35, and 55 °C. All three curves can be described by eq. (1) and yield values of the parameter $\text{Hb}(\text{W})$ increasing from $-65.6 \text{ kJ mol}^{-1}$ at 5 °C to $-34.4 \text{ kJ mol}^{-1}$ at 55 °C.

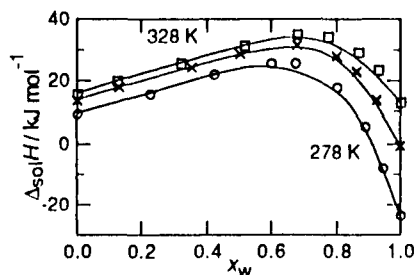


Fig. 9. Enthalpies of solution of $n\text{-Bu}_4\text{NBr}$ in mixtures of water and DMF at 278, 308 and 328 K.

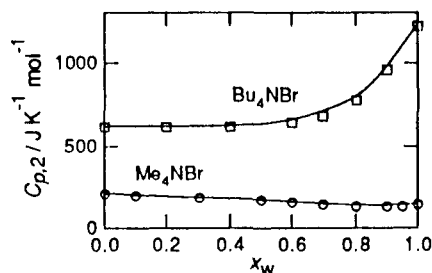


Fig. 10. Partial molar heat capacities of Me_4NBr and $n\text{-Bu}_4\text{NBr}$ in mixtures of water and DMF.

From these enthalpies it is possible to obtain the standard partial molar heat capacities of Bu_4NBr at different compositions. The resulting curve, together with that for Me_4NBr , is presented in Fig. 10. In pure DMF the partial molar heat capacity of Bu_4NBr is close to $600 \text{ J K}^{-1} \text{ mol}^{-1}$ and it remains at that value to $x_w \approx 0.6$. With higher x_w it starts to rise towards its much higher value in pure water, approximately $1200 \text{ J K}^{-1} \text{ mol}^{-1}$. In contrast to this, the partial molar heat capacity of Me_4NBr is virtually independent on composition. Generally, the magnitude of the standard partial molar heat capacity can be considered as the sum of two contributions. The first is the intrinsic heat capacity of the solute particle itself, which would be equal to the standard partial molar heat capacity if solute-solvent interactions were absent. The second contribution is the result of the influence of the solute on the solvent. Hence, Fig. 10 reflects the changes of this second contribution of the two salts. For Bu_4NBr at low x_w , the value of $600 \text{ J K}^{-1} \text{ mol}^{-1}$ is very close to the estimated intrinsic heat capacity of Bu_4NBr and hydrophobic hydration is absent. At higher x_w , solute-solvent interaction becomes significant and in pure water the contribution of hydrophobic hydration is close to $600 \text{ J K}^{-1} \text{ mol}^{-1}$. Also for Me_4NBr the value at low x_w is close to the estimated intrinsic heat capacity. As x_w increases, the partial molar heat capacity becomes smaller than this intrinsic value. Since in pure water the ionic charge contributes in a negative sense, it can be concluded that a possible hydrophobic contribution is either absent or too small to compensate the charge effect.

Normal and isomeric alkanols

Similar differences as for the tetraalkylammonium salts are observed if we compare the enthalpies of transfer of a *non-electrolyte* containing one or more alkyl groups. Also here, transfers between two non-aqueous solvents give results deviating from those between a non-aqueous solvent and water. Figure 11 gives the enthalpies of transfer of n-butanol in mixtures of DMF and NMF and in mixtures of DMF and water. In the non-aqueous mixture all enthalpies of transfer are close to zero. In the aqueous mixtures with a water content lower than $x_w = 0.7$ the enthalpies of transfer are small and endothermic, but in the water-rich mixtures the hydrophobic hydration of the alkanol becomes apparent by a substantial exothermic shift of the enthalpy of transfer towards an exothermic value in pure water. In general, this picture applies for n-alkanols as well as isomeric alkanols. Figure 12 shows the enthalpies of transfer in mixtures of DMF and water for several primary, secondary and tertiary alkanols.

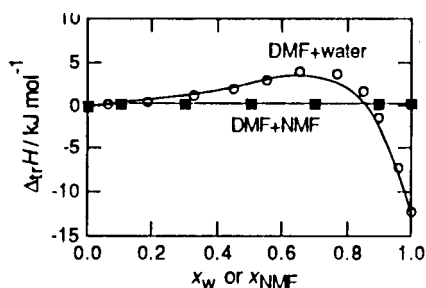


Fig. 11. Enthalpies of transfer of n-BuOH in mixtures of DMF and DMSO with water.

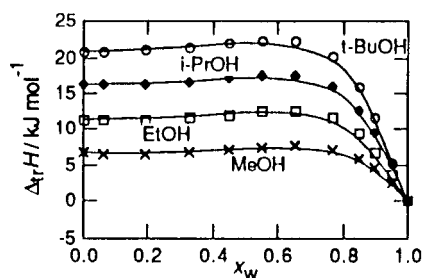


Fig. 12. Enthalpies of transfer of several alkanols from water to aqueous DMF mixtures.

Considering the enthalpies of transfer, $\Delta_{tr}H$, from water to DMF for the different alkanols, it appears that their values in the series methanol, ethanol, secondary propanol, tertiary butanol and in the series n-propanol, secondary butanol, tertiary pentanol show constant increments with an average value of 4.6 kJ mol^{-1} (see Fig. 13). Thus, the subsequent substitution of α -H atoms by methyl groups results in additive contributions to the enthalpies of transfer. This agrees with the idea of independent hydration of groups in the solute molecules already discussed above with respect to tetraalkylammonium compounds. Again it is possible to describe the experimental results in terms of eq. (1). In addition, it can be shown for the alkanols that the values of $\Delta_{tr}H$ are approximately equal to the parameter $Hb(W)$, the enthalpic contribution of hydrophobic hydration in pure water. Figure 13 shows also that the enthalpies of transfer for n-alkanols are always smaller than those of the corresponding isomeric ones

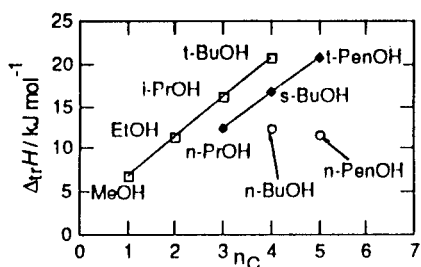


Fig. 13. Enthalpies of transfer of alkanols as function of their number of C atoms.

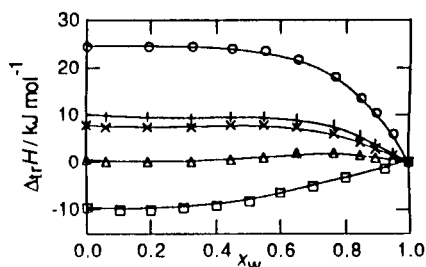


Fig. 14. Enthalpies of transfer of (from top to bottom) tetraMe-urea, 1,3 diMe-urea, 1,1 diMe-urea, Me-urea and urea itself.

suggesting that straight-chain alkyl groups are less effectively hydrated than spherical hydrophobic entities.

Substituted urea compounds

Enthalpies of transfer from water to mixtures of DMF and water for a number of substituted urea compounds are presented in Fig. 14. The parent compound urea is a non-hydrophobic solute and, as Fig. 14 shows, progressive substitution with methyl groups makes the resulting derivatives of urea increasingly hydrophobic. This is reflected also in the enthalpies of transfer from water to DMF presented in Table 2. From this table it appears that the introduction of one methyl group into urea causes a shift in $\Delta_{tr}H$ of 9.67 kJ mol^{-1} . This is distinctly higher than the shift caused by the addition of a second methyl group on the same N atom (to give 1,1-dimethylurea), which is 7.31 kJ mol^{-1} . However, if this second methyl group is introduced at the other N atom (giving 1,3-dimethylurea) the shift is 9.88 kJ mol^{-1} .

TABLE 2. Enthalpies of transfer, $\Delta_{tr}H$, from water to DMF for urea and its methyl substituted derivatives

	$\Delta_{tr}H / \text{kJ mol}^{-1}$	$\Delta_{tr}H / \text{kJ mol}^{-1}$
urea	-9.43	1,1-dimethylurea 7.55
methylurea	0.24	1,3-dimethylurea 10.12
trimethylurea	17.36	tetramethylurea 24.70

The latter value is close to that of the first methyl group, which indicates that the NH_2 and NHCH_3 groups on either side of the molecules are solvated independently. If this is true, it would imply that the shifts in the enthalpies of transfer with respect to urea amount to $9.67 + 9.88 + 7.31 = 26.86 \text{ kJ mol}^{-1}$ for trimethylurea and $9.67 + 2 \times 7.31 + 9.88 = 34.17 \text{ kJ mol}^{-1}$ for tetramethylurea. In excellent agreement with these predicted values, we found experimentally 26.79 and $34.13 \text{ kJ mol}^{-1}$, respectively (ref. 5). Closer inspection of the data underlying Fig. 14 shows that this additivity scheme applies also to the enthalpies of transfer to the mixed solvents. Hence the idea of independent solvation of both sides of the urea compounds is corroborated by the experimental results in the pure solvents as well as in the solvent mixtures.

ENTHALPIES OF SOLUTE-SOLUTE INTERACTIONS IN PURE SOLVENTS

The thermodynamic properties of dilute solutions of non-electrolytes can be described in terms of solute-solvent and solute-solute interactions by means of the McMillan-Mayer theory (ref. 6). On basis of this theory, the excess enthalpy of a solution of two solutes x and y with molalities m_x and m_y can be written as

$$H^E(m_x, m_y) = n_x^2 h_{xx} + n_y^2 h_{yy} + 2m_x m_y h_{xy} + n_x^3 h_{xxx} + n_y^3 h_{yyy} + 3n_x^2 m_y h_{xxy} + 3m_x n_y^2 h_{xyy} + \dots \quad (2)$$

In this equation h_{xx} , h_{yy} and h_{xy} are the enthalpic interaction coefficients for the pairwise interactions between like solute molecules (homotactic interactions) and unlike solute molecules (heterotactic interactions), h_{xxx} , h_{xyy} , etc. the enthalpic triplet interaction coefficients, and so on.

Enthalpic interaction coefficients can be obtained from calorimetric measurements of enthalpies of dilution for homotactic interactions or enthalpies of solution and enthalpies of mixing for heterotactic

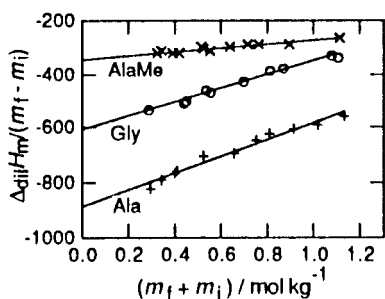


Fig. 15. Enthalpies of dilution of three dipeptide amides (see Table 3) plotted according to eq.(3).

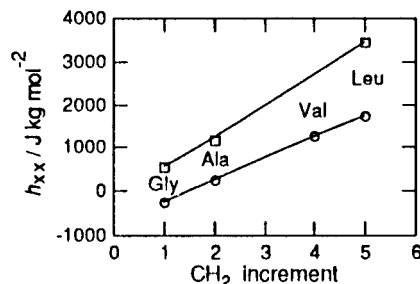


Fig. 16. Enthalpic pair interaction coefficients of methylated (upper curve) and unmethylated (lower curve) dipeptide amides in water.

interactions (ref. 7). In this contribution, the discussion concerns mainly homotactic interactions and will be restricted to enthalpic *pair* interaction coefficients. Values presented have been obtained from dilution experiments with a commercial microcalorimeter. The amounts of heat measured in a single dilution experiment vary from 1 to 250 mJ. Per compound studied, 10 - 20 dilution experiments have been performed covering molalities from 0.1 to 1.0 mol kg⁻¹. The obtained molar enthalpy of dilution, $\Delta_{\text{dil}}H_m$, the initial molality m_i and the final molality m_f are related by

$$\frac{\Delta_{\text{dil}}H_m}{m_f - m_i} = h_{xx} + h_{xxx}(m_f + m_i) + \dots \quad (3)$$

The enthalpic interaction coefficients are determined by a least squares analysis of the experimental data in terms of this equation. Graphically the experimental results can be visualized by plotting the left hand side of eq.(3) with respect to $(m_f + m_i)$. In these plots, the intercept gives h_{xx} and the (limiting) slope is equal to h_{xxx} . Rarely, non-linear relationships are found. Figure 15 gives an example of a graphical representation according to eq.(3) of enthalpies of dilution of some dipeptide amides dissolved in DMF.

Dipeptide-like model compounds

We have studied the enthalpies of interaction of several organic solutes in various solvents. Our aims were to compare enthalpic interaction coefficients in non-aqueous solvents with those in the 'unique' solvent water and to obtain information about biochemical 'non-aqueous' systems, like the interior of globular proteins. Among the factors governing the overall stability of native globular proteins in water hydrophobic interactions of apolar amino acid residues in the water phase may contribute as well as interactions between the amino acid residues in the, almost non-aqueous, interior of globular proteins. Therefore, we performed experiments with model compounds dissolved in model solvents that mimic the protein interior. To avoid the influence of the charges present in amino acids or dipeptides only non-electrolyte solutes have been investigated. Since important molecular entities are the peptide group CONH and alkyl groups of different type, our solutes were mainly substituted amides (ref. 8) with the general structure RCONR'R" (where the substituent may be H) and acetylaminoacid amides (ref. 9) with the general structure CH₃CONHCHRCONHR' (where R as well as R' may be H also). The names of these dipeptide-like compounds and the abbreviations used are given in Table 3.

In order to avoid the introduction of other functional groups in the intermolecular interactions, the non-aqueous liquids adopted as model solvents were NMF and DMF.

Table 4 gives the homotactic enthalpic pair interaction coefficients for some characteristic (substituted) amides and dipeptide amides in the solvents water, NMF and DMF (taken from ref. 8-13). In water, all coefficients are positive and increase in magnitude as the solute molecules contain more and/or larger alkyl groups, thus reflecting the strong influence of hydrophobic interaction. In the non-aqueous solvents all values are shifted in a negative sense with respect to those in water and the order in the values of the enthalpic pair interaction coefficients is reversed. Molecules with more and/or larger alkyl

TABLE 3. Names, structural formulas and abbreviations of the peptide amides.

name	CH ₃ CONHCHRCONHR'		abbreviation
	R	R'	
<i>N</i> -acetyl (<i>N</i> -methyl) glycinamide	H	H, CH ₃	Gly, GlyMe
<i>N</i> -acetyl (<i>N</i> -methyl) alaninamide	CH ₃	H, CH ₃	Ala, AlaMe
<i>N</i> -acetyl (<i>N</i> -methyl) valinamide	CH(CH ₃) ₂	H, CH ₃	Val, ValMe
<i>N</i> -acetyl (<i>N</i> -methyl) leucinamide	CH ₂ CH(CH ₃) ₂	H, CH ₃	Leu, LeuMe
<i>N</i> -acetyl (<i>N</i> -methyl) isoleucinamide	CH(CH ₃)CH ₂ CH ₃	H, CH ₃	Ile, IleMe

TABLE 4. Enthalpic coefficients for homotactic pairwise interactions of several amides and dipeptide amides in various solvents.

solute	$h_{xx} / \text{J kg mol}^{-2}$			solute	$h_{xx} / \text{J kg mol}^{-2}$		
	water	DMF	NMF		water	DMF	NMF
PrA ^a	250	-286	-31	DMF	737		67
NBA ^a	1477	-302	-135	DMA ^a	1081	4	162
Gly	-220	-609	82	GlyMe	585	-322	10
Ala	268	-886	-17	AlaMe	1181	-348	-34
Val	1259	-1432	-313	ValMe		-611	-309
Leu	1714	-1149	-229	LeuMe	3420	-550	-260
Ile		-1918		IleMe		-673	

^a PrA : propionamide; NBA : *N*-butylacetamide; DMA : *N,N*-dimethylacetamide.

groups have more negative values. This agrees with the observation of Yaacobi and Ben-Naim (ref. 14) that alkane molecules dissolved in alkanols attract each other without the large entropy changes which are characteristic for aqueous systems. The adherence of non-polar molecules or molecular groups may lead to a smaller distortion of dipolar interactions between the solvent molecules, corresponding to an exothermic contribution to the enthalpy change. This is equivalent to, with respect to water, negatively shifted enthalpic pair interaction coefficients. The shifts will be larger as the apolar part of the molecule becomes larger, so that the order reverses.

As Fig. 16 shows values of h_{xx} for the dipeptide amides in water depend linearly on the molar mass of the solute. This is not true for the non-aqueous solvents and Fig. 17, which presents results in DMF, indicates that especially the values for the valine compounds are deviating.

It is remarkable that this deviation correlates with that of an affinity parameter for amino acid residues in globular proteins defined by Roberts and Bohacek (ref. 15). From structural data on a large set of proteins, these authors reflect the relative contact frequencies between the side chains in amino acid residues by a parameter R_{ab} . Values of R_{ab} larger than one reveal an intrinsic affinity between side chains a and b, whereas $R_{ab} < 1$ indicates a "repulsive" effect. The correlation between the values of h_{xx} and R_{ab} as given in Fig. 18 for h_{xx} values in DMF, exists also for h_{xx} values in NMF (ref. 9), and NMA (ref. 16).

Influence of chirality

The dipeptide amides discussed above are derivatives of the naturally occurring amino acids which all have the L-conformation. For the pairwise interactions in solution no difference is to be expected between the interactions of two L- or two D-peptides, as they form enantiomeric dimers. However, there should be a difference between the interaction of two L- or two D-peptides, and that of an L-peptide with a D-peptide, because L-L (or D-D) and D-L dimers are diastereomeric. This difference in interaction is called chiral selectivity or chiral recognition. It is important in biochemical processes like enzyme-substrate interactions. In recent work on alanyl-glycine (ref. 17) no differences were observed, probably due to the coulombic forces in the zwitterionic structures which will conceal more subtle interaction

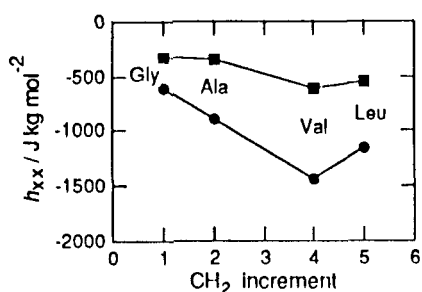


Fig. 17. Enthalpic pair interaction coefficients of methylated (upper curve) and unmethylated (lower curve) dipeptide amides in DMF.

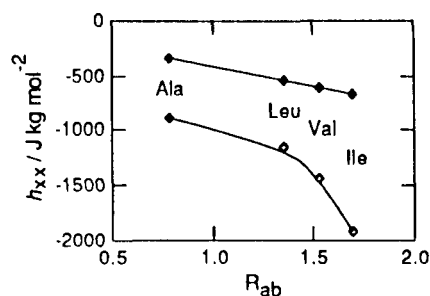


Fig. 18. Correlation between the enthalpic pair interaction coefficients of methylated (upper curve) and unmethylated (lower curve) dipeptide amides and the affinity parameter R_{ab} in DMF.

TABLE 5. Experimental enthalpic interaction coefficients h_{xx} for homotactic pairwise interactions of racemic, D-, and L- dipeptide amides and values for homochiral and heterochiral pairs.

compound	$h_{xx} / \text{J kg mol}^{-2}$			$h_{DD} / \text{J kg mol}^{-2}$	$h_{DL} / \text{J kg mol}^{-2}$
	D	L	(D,L)		
AlaMe	-350	-348	-307	-350	-265
ValMe		-611	-489	-611	-367
LeuMe	-550	-530	-310	-550	-80
IleMe		-673	-537	-673	-403

effects. But later work on non-ionic dipeptide amides in water revealed significant differences between the interactions of solutes with similar chiral centres (homochiral) and solutes with different chiral centres (heterochiral). Table 5 presents the enthalpic pair interaction coefficients of pure and racemic methylated dipeptide amides dissolved in DMF. Within uncertainty limits the coefficients for the homotactic D-D and L-L interactions are equal.

The same is true for water as solvent. For the racemic D-L compounds it can be shown that the experimentally obtained apparent enthalpic pair interaction coefficient is given by

$$h_{xx} = \frac{1}{4}h_{DD} + \frac{1}{2}h_{DL} + \frac{1}{4}h_{LL} \quad (4)$$

Since $h_{DD} = h_{LL}$, the enthalpic pair interaction coefficient for a homotactic heterochiral interaction can be calculated from h_{xx} and h_{LL} . The results for several dipeptide amides are given in Table 4. It appears that heterochiral pairwise interactions are less exothermic than homochiral ones and that the difference depends considerably on the size and the structure of the amino acid side chain. The isoleucine compound, with its side chain branched at the β -carbon atom shows a much smaller difference than the leucine compound whose side chain branches at the γ -carbon atom. In the same way, the proline compound with a five-membered pyrrolidine ring and consequently conformationally restricted, shows a smaller difference than the alanine compound. Hence, conformational aspects are important. In spectroscopic studies it has been shown that in solution these dipeptide amides reside

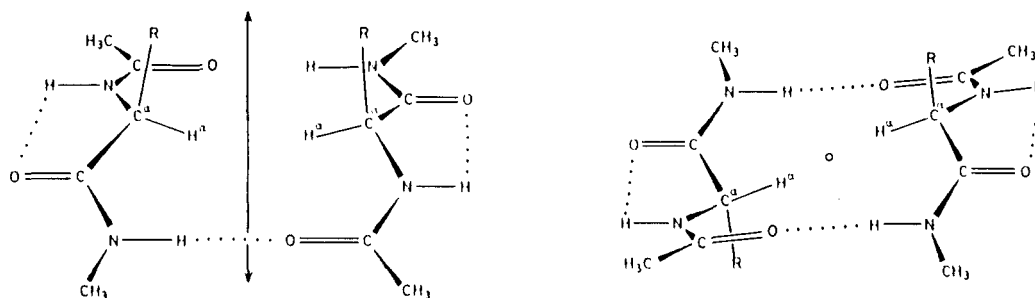


Fig. 19. Homochiral (left) and heterochiral (right) pair of interacting dipeptide amides (see text).

preferably in a folded C₅ or C₇ conformation (ref. 18). With respect to homo- and heterochiral interactions between pairs of these molecules, Cung *et al.* (ref. 19) proposed a model for the structures of dimers as represented in Fig. 19. The two molecules interact by means of two hydrogen bonds. When two homochiral compounds interact, their two side chains, denoted by R, are in close proximity, in contrast with the situation for two heterochiral peptides.

As a result the polarophobic interaction for two homochiral compounds is enhanced and the h_{LL} or h_{DD} coefficients are more negative.

In water, the differences between the (positive) enthalpic pair interaction coefficients corresponding to the homo- and heterochiral interactions are much smaller. For the folding process of globular proteins this seems to imply that subtle conformational variations in the molecules may play an important role.

CONCLUSIONS

With calorimetric techniques it is possible to obtain important information with respect to solute-solvent and solute-solute interactions in solutions. Experimental results on the enthalpies of solution of electrolytic as well as non-electrolytic compounds carrying alkyl groups clearly show the influence of hydrophobic hydration in water and in aqueous solvent mixtures with a high water content. In the mixtures, this influence decreases strongly with diminishing amount of water, so that with respect to solvation aqueous solvent mixtures with less than 70 mole % of water behave as non-aqueous solvents. For solute molecules with different functional groups, the total solvation effects are due to additive contributions from the molecular groups. Microcalorimetric investigations of solute-solute interactions for substituted amides and dipeptide amides reveal the importance of the alkyl groups in the molecules. Enthalpies of interaction in water and in non-aqueous solvents are comparable in magnitude, but differ in sign. Chirality and other conformational details in the solute molecules are very important in non-aqueous solvents. In water, their effects are smaller.

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