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Peptide–peptide interactions in water and concentrated urea solutions

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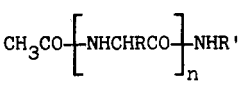
Abstract - A review is presented on some recent developments in the thermodynamics of aqueous solutions of organic substances, that can be considered as models of the repeating units of naturally occurring polypeptides and proteins. The attention is firstly focused on the solute-solvent interactions as basis to understand some aspects of the solute-solute interactions. The solvent in fact, especially in dilute solutions, seems to take active part in these interactions. All these poorly specific interactions, as are repeated for each aminoacid residue, must be considered in the conformational treatments of the stability of proteins. Preliminary interesting data are also reported, concerning model peptide-model peptide and other interactions in concentrated aqueous solution of urea, that is a usual denaturing medium for proteins and biopolymers. The unexpected and stimulating results suggest that, in this solvent, the hydrophilic interactions are screened, probably because polar groups are preferentially solvated by urea. Moreover the highly polar urea-water mixtures seem still to exert a kind of "lipophobic" effect against the alkylic chains of the considered solutes.

INTRODUCTION

It is generally accepted that the biologically active conformation of proteins and other natural macromolecules, for a given primary structure, is unique, thermodynamically stable and resulting from a delicate balance of contrasting intermolecular and intramolecular weak interactions (ref. 1). The peptide model systems then became the subject of many studies since they can give information on the interactions with the solvent and on the long-range intramolecular interactions, that have a role in the folding. The peptide-urea interactions also are of great interest in biophysical chemistry, since they concern the denaturation processes, often used in perturbative approaches to gain an insight in the stability of proteins. To avoid complications due to the presence of pairs of net charges, as in the case of dipolar ions of aminoacids, different kinds of uncharged molecules have been used in the past for peptide-peptide and peptide-urea interaction model studies (refs. 2-7). Only a few data on urea-amide aqueous systems have been published (refs. 8-13), in spite of the fact that also amides can be considered as good models for the structural units of polypeptides. Some years ago Lilley and coworkers proposed to use, as model molecules, the uncharged amides of the N-acetyl derivatives of aminoacids and peptides of general formula:



with n=1,2,3..., R being a side chain of a naturally occurring or synthetic aminoacid and R'=-H, $-CH_3$. The terms with n=1, especially, are very suitable for interaction studies, with respect to uncharged molecules already used, for their good solubility in water. In a long series of papers Lilley and coworkers (for a recent review see ref.14) have studied the dilute solutions of these N-acetylamides of aminoacids. Recently at the author's laboratory the interactions between enantiomeric aminoacid and aminoacids derivatives have been also studied (refs. 15-18).

On the other hand, the knowledge of peptide-water, peptide-peptide and peptide-urea interactions is not sufficient for unravelling the properties of these solutions. It is well known that one of the interactions, commonly considered responsible for the tilted conformation of globular proteins and for other features of biological macromolecules, is the hydrophobic interaction or effect or bond (refs. 19-23). This kind of interaction is still the subject of many controversies, but the studies of Ben-Naim (refs. 24,25), Friedman (refs. 26,27) Franks (refs. 28-31) and coworkers, have contributed to clarify many aspects (one of the main being the distinction of the apolar group-water interaction, i.e. the so called hydrophobic hydration, from the apolar solute-apolar solute interaction, "assisted" or "mediated" by the solvent water). Theoretical contributions often revise critically all the matter (refs. 32-36), so the hydrophobic forces are still the object of extensive studies.

The existence of mixed interactions, between polar and apolar groups, have been stressed some years ago by Savage and Wood, who proposed also an additivity of group method for analyzing all dilute solutions, in a given solvent, of non-electrolytes (ref. 8). Water and aqueous solutions are particularly favourable to this kind of approach (and the Savage and Wood proposal was somewhat successful in the past years), because the role of water as "assisting solvent" to the solute-solute weak interactions probably makes comparable all the solute-solute, solute-solvent and solvent-solvent non bonding interactions.

This review, necessary limited in its purposes, likes to cover recent progress on thermodynamics of dilute solutions of peptides, amides and related solutes and discuss briefly some models proposed to rationalize the properties of these systems. Two experimental approaches are possible and it is useful to couple them for a better understanding of the solution properties: a) hydration or solvation studies, that deal essentially with the transfer of the solute species from ideal gas phase to the infinitely dilute solution (in water or whatever solvent or mixture); b) interaction studies between molecules of the same or different solute species. The discussion will be focused only on the pairwise interactions, unique in that they have a sufficiently defined mechanical statistical basis, the analysis among three or more molecules being on the contrary ill founded. For the interaction studies the role of the solvent cannot be neglected, for the competitive nature of the solute-solvent interactions, with respect to the solute-solute ones, when the solvent is so polar as water. For this reason and because the water-urea mixtures at high concentration of urea are the real denaturing medium of proteins, the interaction studies were extended to this mixed solvent. Preliminary results and extended papers have been just submitted or are in preparation (refs. 37-42).

ENTHALPIES OF HYDRATION AND SOLVATION STATE OF AMIDES

The more complete studies on the hydration of molecules interesting as models for peptides have concerned the liquid amides . The combination of enthalpies of vaporization and solution in water at 298.15 K gives the enthalpies of hydration, i.e. the enthalpies of transfer of the solute from gaseous phase to the infinitely dilute solution:

$$\Delta_{hydr}H^\circ = \Delta_{sol}H^\circ - \Delta_{vap}H^\circ \qquad (1)$$

Such data, both determined calorimetrically, are useful for comparison, as they refer to the ideal vapour as common reference state. The experimental $\Delta_{hydr}H^\circ$ of liquid amides are all negative (Table 1), the absolute values increasing on increasing the lenght of the alkylic chains. The data can be analyzed according to a group additivity approach:

$$\Delta_{hydr}H^\circ(x) = \sum n(A) \cdot \Delta_{hydr}H^\circ(A) \qquad (2)$$

where n(A) indicate the number of each group of atoms (A) into which is ideally divided the substance x. The different amidic groups ($-CONH_2$,$-CONH-$, $-CON=$) and the methylene equivalent group (CH_3 being considered as 1.5 CH_2 and CH as 0.5 CH_2) are necessary to describe the data. It is possible to see that the contributions of the alkylic groups to the enthalpies of hydration are -6 kJ/mol (CH_2) for monosubstituted and -4.5 kJ/mol (CH_2)

for disubstituted amides, in agreement with results concerning other solutes bearing normal or branched alkyl chains (refs. 46,48,50,51). This effect arises from the hydrophobic surface exposed to the aqueous solvent. The proximity of alkylic chains explains the little difference found in the case of disubstituted amides. This kind of "hydrophobic" (refs. 24-30) or "aperipheral hydration" (ref. 52) must be distinguished from the "hydrophobic effect" pertaining to solute-solute interactions. The negative sign of these enthalpic contributions are a proof of the rearrangements of water-water interactions in the cosphere of the alkylic chains that overwhelm the cavitation term.

TABLE 1 - Enthalpies of solution in water, vaporization and hydration for amides at 298.15 K.

Compound	$-\Delta_{sol}H°$	$\Delta_{vap}H°$	$-\Delta_{hydr}H°$
F	-2.03	60.1	58.1
NMF	7.7	56.25	63.9
NEF	9.7	57.9	67.6
DMF	16.3	46.9	63.2
DEF	19.6	50.3	70.0
DPF	20.7	55	75.7
A	-9.74	80.3	70.6
NMA	3.84	69.9	73.3
NEA	15.48	64.9	80.4
NPA	15.76	69.8	85.5
NBA	14.72	75.0	89.7
DMA	21.42	50.2	71.7
DEA	25.0	54.1	79.1
NMP	14.87	64.9	79.8
DMP	22.4	52.9	75.3
NMB	16.02	69.8	85.8
NMPe	15.03	75.0	90.0

Units: kJ/mol. Data from refs. 43-49.
Abbreviations: F:formamide; NMF:N-methylformamide;NEF:N-ethylformamide;DMF:N,N-dimethylformamide; DEF: N,N-diethylformamide; DPF:N,N-dipropylformamide; A: acetamide; NMA: N-methylacetamide;NEA:N-ethylacetamide; NPA:N-propylacetamide; NBA:N-butylacetamide; DMA:N,N-dimethylacetamide; DEA:N,N-diethylacetamide; NMP: N-methylpropionamide; DMP:N,N-dimethylpropionamide; NMB:N-methylbutyramide; NMPe: N-methylpentanamide.

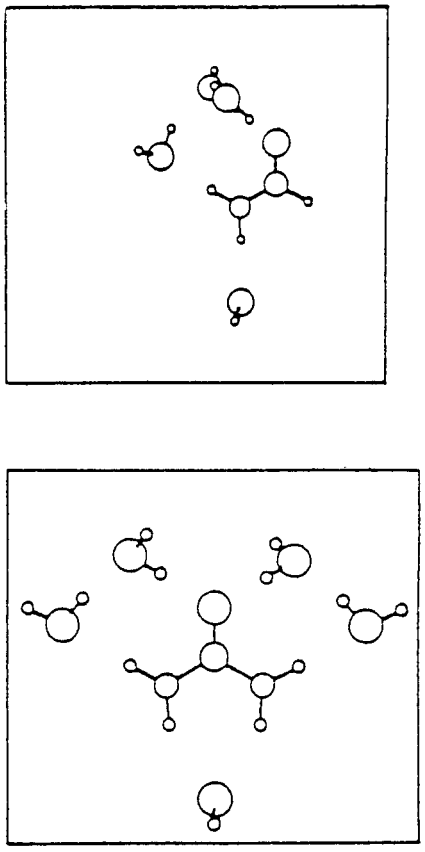


Fig.1. Structure of the first hydration shell of formamide (above) and urea (below). (Ref.54)

As expected, the hydration enthalpies are more negative for each monosubstituted amide than for the corresponding isomeric dialkylamide, because of the number of hydrogen bonds that the groups can form with solvent water. The values of -58.1 kJ/mol for FA and of -70.6 for AA suggest that the formation of at least four new hydrogen bonds (ref. 53) are induced by the amidic group (besides other dipolar and van der Waals contributions). Recent calculations (ref. 54) based on the energy minimization of clusters of one FA molecule and an increasing number of water molecules up to 11, allowed to assign a first hydration shell of four water molecules to the FA (Fig. 1) Following the SCSSD model (ref. 55) a solvation enthalpy of -50 kJ/mol was calculated for FA taking into account all the interactions in the minimum energy configuration of the cluster and bulk. Likewise, five molecules were assigned to the first hydration shell of urea (Fig. 1). The group contribution estimate gives a value of about -59 kJ/mol for the hydration enthalpy for the amidic groups, of about -50 kJ/mol for peptidic groups of monosubstituted formamide derivatives, and values between -55 and -61 kJ/mol for peptidic groups of other monoalkylamides, depending on the lenght of the alkylic chains. The values of the solvation enthalpies for the -CON< groups

are estimated to be about -44 kJ/mol for the dialkylformamides and about -49 kJ/mol for other dialkylamides, independently from the lenght of alkylic chains (ref. 44).

HYDRATION OF PROTECTED AMINOACIDS AND UREA

The determination of the hydration enthalpies of protected aminoacids, as N-acetylamide derivatives, offers some difficulties, because the vaporization rate of these solids is very low at 298.15 K. A research programme, recently begun, was finalized to the determination of the sublimation enthalpies, obtained from the temperature dependence of the vapour pressures. These were measured by means of a torsion-effusion method. Because the sublimation process was carried on at temperatures much higher than 298.15 K, it is needed to have at disposal (to convert the data at standard temperature) the integrated values of the C_p of the solids and vapours over the entire temperature range. The enthalpy of hydration then can be obtained from the relationship:

$$\Delta_{hydr}H^{\circ}_{298} = \Delta_{sol}H^{\circ}_{298} - \Delta_{sub}H_T - \int_{298}^{T} C_p(s)dT - \int_{T}^{298} C_p(g)dt \qquad (3)$$

More steps will be needed, if solid state transitions are present. In this case each transition enthalpy must be taken into account and the integral of the third term on l.h.s. of the eqn. (3) must be substitued with a sum of integrals. In Table 2 the enthalpies of solution in water at 298.15 K and those of sublimation, at the given temperature ranges, are reported for some N-acetylamides of aminoacids and two cyclic anhydrides of aminoacids (diketopiperazines) (refs. 56-59). Enthalpies of solutions for N'-methylated acetylamides have been determined recently (ref. 60). The values for urea are also reported (ref. 61). In all cases no solid state transitions were detected. In the same Table 2 the differences between the enthalpies of solution and sublimation are reported. In the case of N-acetylamides it was possible to estimate the values of the hydration enthalpies from theoretical calculations of $C_p(g)$ (ref. 93) and preliminar experimental determinations of $C_p(s)$. Work is in progress for refining and extending this approach to other systems. The detailed knowledge of the crystal packing is indispensable for explain the anomalies in the sequences of properties of solids with respect to the molecular weights and number of alkylic and functional groups (refs. 58,62-65).

TABLE 2. - Enthalpies of solution, sublimation and estimated enthalpy of hydration for N-acetylamides and cyclic anhydrides of aminoacids.

	$\Delta_{sol}H^{\circ}_{298}$ (kJ/mol)	$\Delta_{sub}H_T$ (kJ/mol)	Subl. range (K)	$\Delta_{sub}H^{\circ}_{298}$ (kJ/mol)	$\Delta_{sol}H^{\circ}-\Delta_{sub}H_T$ (kJ/mol)	$\Delta_{hydr}H^{\circ}_{298}$ (kJ/mol)
NAGA	15.89	135	378-408	139.3	-	-123.4
L-NAAA	5.53	115	366-411	119.7	-	-114.2
L-NAVA	9.37	125	394-445	130.7	-	-121.3
D-NALA	0.44	101	374-401	102.5	-	-102
L-NAPA	5.03	135	360-403	-	-131.5	-
cG_2	26.38	155	440-516	-	-128.5	-
$cSar_2$	4.02	107	341-406	-	-103	-
Urea	15.26	89	354-409	-	-74	-

Abbreviations: NAGA, N-acetylglycinamide; NAAA, N-acetylalaninamide; NAVA, N-acetylvalinamide; NALA, N-acetyl leucinamide; NAPA, N-acetylprolinamide; cG_2, cyclic glycilglycine anhydride; $cSar_2$, cyclic sarcosylsarcosine anhydride.

EXCESS THERMODYNAMIC PROPERTIES

The excess thermodynamic properties deal with the deviations of the systems from the ideality. For treating real liquid mixtures, rational activity coefficients and pure component standard states are conveniently used. For unsymmetrical dilute solutions, in particular of metabolytes and their models, most of them solids at room temperature, a different choice is useful for the standard state (pure solvent and solute at infinite dilution). For practical reasons the experimentalists use the molality scale, all the trasforming relationships being known (ref. 66). Kauzmann (ref. 67) and Friedman (ref. 27) adapted the McMillan-Mayer theory of solutions (ref. 68) to the aqueous solutions of uncharged organic molecules and other nonelectrolyte mixtures. An excess thermodynamic property, J^E can be defined per each osmolality as follows:

$$J^E = J - J^\circ(1) - \sum_{x=2}^{n} m(x)\,\bar{J}^\circ(x) - J^{ID} \qquad (4)$$

where J is the solute property, $J^\circ(1)$ the value of the property for 1 kg of the pure solvent, $\bar{J}^\circ(x)$ the limiting partial molal quantity of each solute x, m(x) the corresponding molality and J^{ID} the ideal term. A virial-type power expansion series is often used to express each excess thermodynamic property as a function of the molality of the solutes:

$$J^E = \sum\sum j(xy)m(x)m(y) + \sum\sum\sum j(xyz)m(x)m(y)m(z) + \text{higher terms} \qquad (5)$$

In principle the coefficients of the excess Gibbs free energies -g(xy), g(xyz) etc.- will represent the interaction coefficients relative to pairs, triplets and higher number of solute particles. The classical thermodynamic transformations will relate the second enthalpic, entropic, volumetric etc. coefficients to that of the free energy. The physical meaning of the coefficients of eqn. (5) however is much more complex. This is clear considering the second virial coefficients of the osmotic pressure B*(xx) and internal energy u(xx), that have a simpler statistical mechanical meaning. For spherical solute molecules it results:

$$B^*(xx) = -1/2\int_0^\infty \left\langle \left[\exp(-W(r)/kT)-1\right] \right\rangle 4\pi r^2 dr \qquad (6)$$

and

$$u(xx) = 1/2\int_0^\infty \frac{\partial\langle W(r)/kT\rangle}{\partial(1/kT)} \cdot g(r)\, 4\pi r^2 dr \qquad (7)$$

where W(r) is the mean force potential, g(r) the pair correlation function and the quantities in the $\langle\ \rangle$ are the result of an averaging procedure on all the possible orientations of the solvent molecules. For non-spherical solute molecules it needs to introduce a mean force potential $W(r, \Phi_k)$, where $\Phi_k = (\varphi_1 \ldots \varphi_k)$ is a set of angles defining the reciprocal orientation of two solute molecules, and to proceed to a multiple integration over the new set of variables, or to make an average on all the possible orientations of two solute molecules. These mechanical statistical definitions clarify that in principle the excess thermodynamic properties do not depend exclusively on the solute-solute interactions, but also on the changes, with respect to the reference state (i.e. the infinitely dilute solution), of the solute-solvent and solvent-solvent interactions. Only in the case that solute-solute interactions are largely predominant, the other interactions can be neglected, but that seems not to be the case of aqueous solutions of uncharged organic molecules. Introducing in the McMillan-Mayer-Kauzmann-Friedman formalism a dimerization model, it can be shown that it is: $g(xx) = -RTK_D$; $h(xx) = K_D \cdot \Delta H_D$. Physically significant values of K_D, and ΔH_D can allow to distinguish the associating solutes from those weakly interacting. Besides the absolute values of the coefficients (that can differ by two or three order of magnitude or more) must be considered. Observing the known excess properties at 298.15 K of organic substances in water, it was proposed (ref. 69) that most of the not associating solutes can be phenomenologically divided in three g oups according to the signs of the second virial coefficients:

prevailingly hydrophobic solutes	$g(xx) < 0$; $Ts(xx) > h(xx) > 0$
hydrophilic (urea-like) solutes	$g(xx) < 0$; $h(xx) < Ts(xx) < 0$
hydrophilic (sucrose-like) solutes	$g(xx) > 0$; $h(xx) > Ts(xx) > 0$

To the first set pertain alcohols and other solutes bearing one or more aliphatic short chains (not the surfactants); the second set encloses hydrophilic solutes that perturb the ordered local structure of the water (chaotropic solutes), essentially because their orienting effect is not compatible with an ice-like organization of the solvent; the third set encloses essentially oligosaccharides: the solute-solvent interactions are probably dominant for these substances and screen the other ones.

The predominant interactions, characterizing the first set of solutes are the hydrophobic interactions, already discussed in the Introduction, that have been envisaged as an entropically driven overlap of the ordered cospheres of apolar frameworks of solutes molecules, with releasing of water to the bulk (refs. 24-30). In other words the perturbation induced by the apolar solute in the solvent water at infinite dilution, will increase less than proportionally with the solute concentration. Likewise, it was proposed for urea-like solutes (refs. 69,70) that the coalescence of "distorted" cospheres is responsable for the excess thermodynamic properties, rather than a direct solute-solute interactions. The last point is of noticeable interest to rationalize the results of the next sections, concerning peptide-peptide interactions, because the dimerization model of urea was very popular for a long time (refs. 71-73). Really spectroscopic (refs. 53,74-77) and thermodynamic (refs. 69,78) indications support very much the coalescence model. Recent simulations with molecular dynamics have shown that the hydrogen-bond betweeen two urea molecules, placed in a cluster of water, breaks after about 50 psec, the two molecules assuming a different orientation that favours a network of interactions. The two ureas so will interact through chains of hydrogen bonded water molecules and by means of residual van der Waals interactions, even if remaining neighbours (ref. 79). Energy minimization approach to the assemblage of clusters of two urea or FA molecules and a limitated number of water molecules, confirm these conclusions (ref. 54) (Fig.2).

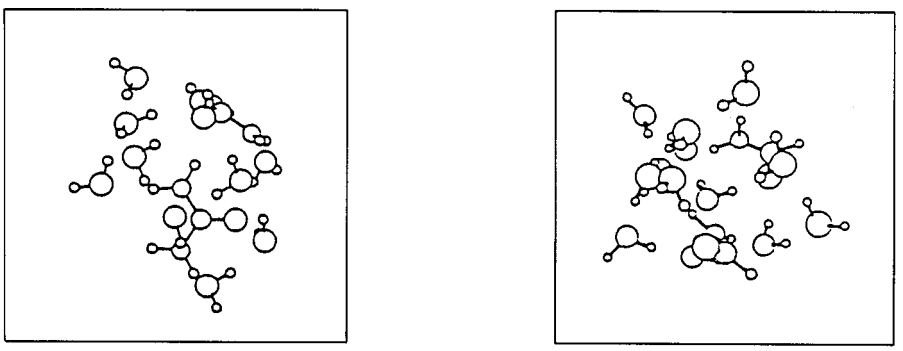


Fig.2. Structure of the fully optimized cluster U_2-W_{10} (left) and $(FA)_2$-W_{11} (right). (Ref.54)

PEPTIDE-PEPTIDE AND OTHER INTERACTIONS IN WATER

In Table 3 the second virial coefficient of Gibbs free energies, enthalpies and entropies at 298.15 K are reported for N-acetylamides and anhydrides of aminoacids, for urea and some of its derivatives and for some amides. The more hydrophilic compounds behave as urea-like, at least at this temperature, the other ones as prevailingly hydrophobic. Monomethylurea shows an intermediate behaviour. For the second group the values of the coefficients increase approximately with the square of the number of methylene groups per solute molecule. The absolute values for the first group seem also to increase rapidly with the number of functional groups.

The cross enthalpic coefficients h(xy) that characterize the interactions between N-acetylamides of different aminoacids and peptides were reviewed in ref. 14. Many of the results comply approximately with the combinatorial square-root rule: $h(xy) = [h(xx)h(yy)]^{1/2}$ (refs. 69,82). The data concerning the N-acetylamide of phenylalanine (NAFA) outline vice versa the existence of weak but specific and favourable interactions especially for the NAFA-NAGA pair. Protected dipeptides, differing from each other for sequences of aminoacid residues, show remarkable differences in the h(xy) values. These results outline that

predictions on the properties of biopolymers are hazardous. Other examples of weakly specific interaction are reported in Table 4 , where the interactions between pairs of homotactic and heterotactic enantiomers are compared (refs. 15,16). Only the enthalpic second coefficients are at our disposal and they outline the existence of a kind of chiral recognition. Other data are given in refs. 14 and 83.

A comprehensive approach that allows to analyze the data all together and stress the existence of mixed interactions (between polar and apolar regions of pairs of solute molecules), was the group additivity method of Wood and coworkers (refs. 8,80). Each second virial coefficient of the excess thermodynamic properties, j(xy), was formally factorized in a sum of group contributions J(AB), extended over all the pairs of groups of atoms A and B composing the two interacting molecules (of the same or different species):

$$j(xy) = \sum_{AB} n(A,x)\, n(B,y)\, J(AB) \qquad (8)$$

The choice of the group of atoms is arbitrary and Wood suggested to minimize the number of parameters by means of empirical assumptions (f.i.: CH = 0.5 CH_2 , CH_3 = 1.5 CH_2 ; $CONH_2 \equiv$ CONH $\equiv$ CON, etc.). The method was often the subject of criticisms (f.i. see ref. 84). However it works approximately for aqueous solutions of simple solutes, where only weak non-bonding solvent mediated interactions occur. This is a reasonable indication that in these systems all the group interactions are probable and there are not present predominant associated species of fixed configurations. Due to the statistical nature of the approach, the group additivity requires a large basis of experimental data. It is difficult to have at disposal a set of systems with very few functional groups. Considering otherwise all kinds of small organic solutes the number of parameters increases rapidly.

TABLE 3 - Second virial coefficients of excess thermodynamic properties in water at 298.15 K for N-acetylamides and cyclic anhydrides of aminoacids, and for amides and urea derivatives (from refs. 4-16, 69,70,78,80-82). Units:(J/mol)/(mol/kg). Abbreviations: see Table 1 for amides (and ureas) and Table 2 for N-acetylamides (M indicates a further methyl group.)

	-g(xx)	h(xx)	Ts(xx)		-g(xx)	h(xx)	Ts(xx)
NAGA	83	-220	-137	FA	38	-115	-77
NAAA	114	273	387	AA	123	12	135
NAVA	-	1259	-	PA	113	249	362
NALA	732	1969	2701	NMF	-	272	-
NAGAM	77	585	662	NMA	356	236	592
NAAAM	119	1181	1300	NMP	-	636	-
NALAM	681	3420	4101	NBA	-	1477	-
NAFA	-	1049	-	DMF	84	737	821
NAPA	111	660	771	DMA	1160	962	2122
NASarA	90	145	235	Thiourea	313	-970	-657
NMAAA	171	587	758	Urea	84	-350	-266
NAPAM	174	1763	1937	MMU	111	-85	26
NASarAM	-	1417	-	MEU	249	160	409
NAG_2A	121	-646	-525	MPU	484	292	776
NAA_2A	200	939	1139	1,1DMU	235	38	273
NAP_2A	241	2010	2251	1,3DMU	247	35	282

TABLE 4 - Enthalpic second virial coefficients for D- and L-NAAA and NALA in water at 298.15 K. Units: (J/mol)/(mol/kg). From refs. 15 and 16. In parentheses the 95% confidence limits are given.

	NAAA	NALA
h(DD)	278(5)	1919(28)
h(LL)	273(5)	1969(24)
h(DL)	294(5)	1822(41)

TABLE 5 - Enthalpic pairwise coefficients for cross interactions between urea and uncharged aminoacids derivatives in dilute solutions at 298.15 K. Units: (J/mol)/(mol/kg). In parentheses the 95% confidence limits. The large error for NALA is due to the presence of the other coefficients. (In part from ref. 18).

	h(xy)	$n(CH_2)$
NAGA-U	-439(10)	2.5
NAAA-U	-329(23)	3.5
NAGAM-U	-269 (6)	4
NAAAM-U	-66 (2)	5
NAPA-U	-339 (5)	5
NAVA-U	-73 (6)	5.5
NALA-U	-124(47)	6.5

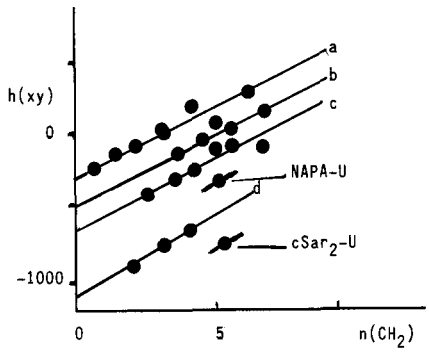


TABLE 6 - Group contributions to the second virial coefficients of the excess thermodynamic properties in water for protected aminoacids (pep), amides (am) and ureas at 298.15 K. Units: (J/mol)(mol/kg).

	G(xy)	H(xy)
CONH-CONH(am)	-	-260
CONH-CONH(pep)	-50	-292
CONH-CONH(cis)	-	-430
CON-CON(pep)	-62	-319
U-CONH(am)	-54	-302
U-CONH(pep)	-54	-376
U-CON(am)	-54	-501
U-CONH(cis)	-	-501
U-CON(pep)	-	~ -555
U-CON(cis)	-	~ -665
CH_2-CH_2(am)(pep)	-20	25
CH_2-CH_2(ureas)	-31	25
U-CH_2(am)	2	101
U-CH_2(pep)	2	123
U-CH_2(cyclic pep)	-	121
CH_2-CONH(pep)	22	80
CH_2-CON(pep)	37	49
CH_2-CONH,CON(cis)	-	90

Fig.3. Coefficients h(xy) for aqueous solutions of: a) amides and monoalkylamides; b) dialkylamides; c) N-acetylamides, d) cyclic anhydrides of aminoacids.

Focusing the attention on peptide-like species, Lilley's and the author's groups were successful in refining the results of Wood. It was possible to distinguish interactions involving a peptide group in cis conformation (ref. 6). Similarly the contributions due to interactions involving completely alkylated CON groups revealed to differ by small but significant amounts from those involving the peptidic or amidic groups (ref. 84). Reconsidering separately the results for the amides it was possible to show that the interactions between the CONH groups on alkylamides gave enthalpic contributions differing (refs. 12,85) from those found in the case of protected aminoacids (ref. 84). Finally studying sets of aqueous systems containing only urea and an amide (refs. 12,13) or a cyclic anhydrid (refs. 5,6) or an acetylamide of simple aminoacid (ref. 18 and this work) it was possible to distinguish among the interactions of urea with the same polar groups placed in different molecular environments. In Table 5 the results concerning aminoacid derivatives and urea, partly unpublished, are summarized. All the results concerning amides, protected aminoacids and urea are shown in Fig. 3 as function of the number of equivalent methylene group of the peptide-model species. The results are grouped clearly (with a few exception) in four families that all show a linear dependence on the methylene number. That is a proof of the empirical validity of the additivity of groups. Separate fittings for each family give refined value for the H(U-CONH) and H(U-CH_2) parameters.

In Table 6 are summarized those and other refined results of the group approach. For the Gibbs free energies the few experimental results allow to distinguish only between the G(CONH-CONH) and the G(CON-CON) contributions. In any case the unsubstituted and monosubstituted amidic groups behave similarly. Differences in the H(AB) values are found when two polar groups are present on a given solute molecule (alternate with alkylic groups): this fact seems to produce a slight cooperative contribution. Considering all the

systems together, the data are forced to fit a line with an underestimated slope and a too high standard deviation.

Some results are of particular interest: a) the peptide-peptide and hydrophobic (methylene-methylene) interactions are both favourable and their unspecific contribution must be taken into account as favourable to intramolecular contacts and folding, in the case of globular proteins. Vice versa ,the peptide-methylene interactions seem to be unfavourable; b) the urea-peptide interactions are favourable. Then urea will compete with intramolecular peptide-peptide interactions and promote (at high concentration) the unfolding of proteins. The urea-methylene interaction seems unfavourable, then it does not promote the unfolding. A surprising result, shown in Table 6 is the fact that completely substituted CON groups exibit a self-interaction and an interaction with urea more favourable than the corresponding interactions involving CONH groups (refs. 12,13,84). That is an indirect but important proof that these interactions are assisted by water. The interaction with urea can have some consequence for proteins of high proline content. Vice versa, the particularly favourable CON-CON interaction seems rather an effect confined to the dilute aqueous solutions, where the assistance of the solvent is effective, and will not contribute to the interpeptide interactions in globular proteins.

PEPTIDE–PEPTIDE AND OTHER INTERACTIONS IN CONCENTRATED AQUEOUS SOLUTION OF UREA

The concentrated aqueous solutions of urea are the real denaturing medium of proteins and other biopolymers. The knowledge of the changes undergone by the biophysical properties of model molecules in that solvent is important for a better understanding of denaturation mechanism. Moreover the urea-water mixtures are liquids of great physical chemical interest because the ice-like structure is completely destroyed, but mixed hydrogen bonded clusters are still possible. The results of preliminary calorimetric measurements here reported are promising for successful extended thermodynamic studies. The McMillan Mayer approach can be easily adapted to the thermodynamics of solutions in mixed solvents. Eqns. (6) and (7) really are of large generality and represent the basis for treating solute-solute interactions in presence of a mixtures of solvent species. The physical meaning of the virial coefficients is the same as in a single pure solvent. It must be only remembered that the solvation processes are of double nature and in competition each other.

In Table 7 the values of the h(xx) coefficients determined in 7M aqueous urea for N-acetylamides and cyclic anhydrides of simple aminoacids are compared with the values in water. In the same Table are also reported the preliminary data, concerning amides in 8M aqueous urea, published by Lilley (ref.14), and those for alkanols (ref. 40) and diols (ref. 39). All the coefficients are found to be positive in concentrated urea. This is of great interest suggesting that for the more polar compound - FA, NAGA, cG_2, whose h(xx) values in water are negative - the favourable water assisted interactions between the polar groups are cancelled. That is the main feature of these results and can be a proof that urea at high concentration interacts preferentially with the polar groups of the solute (urea fills much more than 50% of the volume at the used concentrations). Because urea is more polar than water (and in principle it can form two hydrogen bonds with an amidic or a peptidic group) it can be hypothesized that urea releases from the solvation shell during the concentration process going from an environment (the cosphere) more rich of interactions, to another (the bulk) less rich of interactions, then giving a positive contribution to the excess enthalpy.

An analysis of the results given in the Table 7 suggests that the data, corrected empirically for the differences in urea concentrations, can fit, with tolerable confidence, a straight line when reported as function of the the square of the number of the equivalent methylene groups. The slope [42±4 (J/mol)(mol/kg)] is a measure of the methylene-methylene interactions in presence of urea. This result is unexpected. In fact the value of $H(CH_2\text{-}CH_2)$ in water is lower, for peptides, ureas and amides [25 (J/mol)/(mol/kg), (Table 5)]. The same occours for mono- and polyalcohols [35 (J/mol)(mol/kg),(ref. 91)]. Moreover the slope for the amides, considering the original values in 8M urea, gives [57 (J/mol)(mol/kg)]. A question rises if the hydrophobic

interactions are still active in concentrated urea, contrary to what is commonly assumed. Free energy data are indispensable to support a definite conclusion. More data, in concentrated urea, are also necessary for trying a group contribution analysis on an adequate statistical basis. The preliminar results confirm that polar group-polar group interactions are screened and that a small positive contribution could arise from mixed interaction between polar and apolar groups. It can be now hypothesized that the preferential solvation of urea on the polar groups will confine water into the proximity of the apolar residues. The alkylic groups, in other words, will be still hydrated, not because of a remarkable attraction between these groups and water, but rather because such a disposition is energetically more acceptable on the whole. Urea, for its own geometry, cannot participate in cages surrounding the apolar groups, as water. In conclusion it seems that even though the chaotropic nature of urea promotes the disruption of ice-like clusters of water, the highly polar mixture of the two cosolvents probably maintains a kind of organization. It can be envisaged that the apolar groups would be expelled from the networks of transient H-bonds and statistically juxtaposed by each other. Because the solvent-solvent interactions are the driving forces that promote these thermodynamic macroscopic effects, the term "lipophobic effect" seems more appropiate (for all the hydrogen bonded solvents) than the used "hydrophobic".

TABLE 7 - Enthalpic second virial coefficients h(xx) for N-acetylamides and cyclic anhydrides of aminoacids, amides, alkanols and diols in aqueous solutions of concentrated urea at 298.15 K. Units: (J/mol)/(mol/kg). (From refs. 14,37-41. Results in water from refs. 8,9,11,13,15,16,82,86-90). D at the end of a compound signifies diol.

	h(xx)/U7M	h(xx)/W
NAGA	290(22)	-220 (9)
NAAA	624(10)	273 (5)
NAVA	1101(36)	1259(44)
NALA	1433(20)	1969(28)
NAIA	2300	2000
NAPA	892(16)	660(28)
cG_2	69 (5)	-1138
$cSar_2$	1412(17)	577

	h(xx)/U8M	h(xx)/W
FA	32 (4)	-115 (2)
AA	157 (5)	1(14)
NMF	352 (3)	272 (2)
NMA	538(24)	236(11)
DMF	810(22)	737 (7)
DMA	1060(47)	1081(28)

	h(xx)/U7M	h(xx)/W
MeOH	167 (6)	224(3)
EtOH	238 (6)	243(10)
iPrOH	278 (6)	339
nPrOH	513 (6)	559(14)
iBuOH	743(41)	1000
sBuOH	874 (7)	916
tBuOH	639(81)	656(33)
nBuOH	846(24)	1245(11)
nPeOH	1190(25)	1724(25)

	h(xx)/U7m	h(xx)/W
EtD	322(28)	415(30)
1,2PrD	431 (6)	593(13)
1,3PrD	324 (7)	523 (9)
1,2BuD	767 (4)	923 (5)
1,3BuD	441(18)	750 (6)
1,4BuD	520 (9)	787 (1)
2,3BuD	577 (7)	837(10)
1,5PeD	741(43)	1335(25)
1,6HxD	1032(22)	2402(35)

Undoubtly, the h(xx) values for mono and bifunctional alcohols, are more positive in water than in urea. However the group additivity analysis shows that in water, besides a negative H(OH-OH) contribution, positive H(OH-CH_2) contributions are present, comparable in value and number to the methylene-methylene contributions. All those are negligible or very small in concentrated urea, so that only the lipophobic interactions contribute to the h(xx) values. The same kind of explanation could be valid for the less polar acetylamides (NAVA and NALA) even if it cannot be excluded that for the more heavy alkylic chains an inversion of the trend with respect to water can occour. At present it seems that, at least for shorter alkylic chains and considering only enthalpy, the lipophobic interactions result more effective in presence of high concentration of urea than in pure water. That is in agreement with the consequences of the Ben-Naim model of hydrophobic interactions, on the solubility of methane and ethane in urea-water mixture (ref. 92).

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