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Aqueous Solutions Containing Amino Acids and Peptides

Part 16.—Solute–Solute Interactions in Solutions Containing some *N*-Acetyl-*N'*-methylamino Acid Amides

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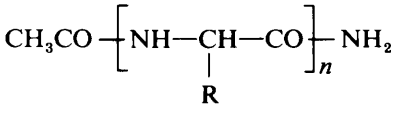
The energetics of the interactions occurring between some *N*-acetyl-*N'*methylamino acid amides in aqueous solutions at 25 °C have been investigated. Osmotic coefficients and enthalpies of dilution have been obtained for single-component and two-component solute systems for the species *N*-acetyl-*N'*-methylglycinamide, *N*-acetyl-*N'*-methyl-L-alaninamide and *N*-acetyl-*N'*-methyl-L-leucinamide. Enthalpy-of-dilution data were also obtained for the sparingly soluble substance *N*-acetylglycyl-L-alaninamide. The results obtained have been used to calculate the pairwise free energy and enthalpy coefficients for the homotactic and heterotactic interactions and these are compared with results obtained earlier on some analogous systems containing the primary amino acid amides. A group-additivity approach has been used to rationalise the information obtained. This works tolerably well for most of the systems investigated.

In some recent papers[1–3] experimental results were presented which related to the interactions occurring in aqueous solutions of some peptides with the general formula



(I)

where R = H, CH_3, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $n = 1-3$.

It was shown that the sociative[4–6] interactions could, in all but one instance, be rationalised in terms of a group-additivity[7–19] approach which represented the nett interaction between two solute species as the result of additive contributions from defined groups. In this regard the group-additivity approach has the same potential utility for intermolecular events as bond-energy procedures have for situations involving covalent bonding. This is not to say that the relative precision is as good for intermolecular effects as for intramolecular effects, but the motivation is the same, namely the estimation of the energetics of an unknown system using rules deduced from systems for which experimental evidence is available.

One of the major difficulties in the study of solute–solute interactions is that they are usually weak compared with thermal energies. This leads to a problem in the interpretation of experimental results in that the rationalisations which are often used in discussions of covalent-bond effects may not be applicable to solute–solute interactions. 'Chemical' interpretations of covalent bonding are usually based on experience, both experimental and theoretical, or intuition. However, for solute–solute interactions there is little experimental evidence on which experience may be based. In the same regard, although there is currently some activity[20–24] on the theoretical front into the behaviour of solutes in aqueous systems, most of this (with some notable

exceptions)[20–26] is concerned with the interaction of isolated solutes with solvent. At the present time it would seem[27] that meaningful and sufficiently accurate calculations on solute–solute interactions in aqueous systems are not possible, although there is no doubt that this situation will change quite rapidly.

One of the principal objectives of the present programme is to obtain experimental results on the energetics of the interactions occurring between amino acids, peptides and some of their derivatives. Our hope is that a body of knowledge may be constructed which initially might lead to the formulation of rules delineating the major contributions to the interactions. The molecular species investigated have the advantage that relatively small and subtle changes in the molecular structures can be made, *e.g.* substitution of a methyl group for a hydrogen atom to give an alanyl rather than a glycyl derivative. The peptides also have the added advantages of allowing the investigation of both sequence dependence and chirality. Investigations such as these bear on the general problem of 'non-bonding' interactions. Our particular interest in peptides stems from their importance in such areas as protein folding, peptide binding to enzymes and cellular receptors and other aspects of molecular recognition.

The present experimental work is a fairly straightforward extension of the studies referred to earlier[1–3] in that we have synthesised some *N*-acetyl-*N'*methylamino acid derivatives

$$\begin{array}{l} CH_3CONH\ CH\ CONH\ CH_3 \\ \quad\quad\quad\quad\ \ | \\ \quad\quad\quad\quad\ \ R \end{array} \qquad \text{(II)}$$

where R = H, CH_3 or $CH_2CH(CH_3)_2$ and have also investigated the Gibbs free energies and enthalpies of both homotactic (like–like) and heterotactic (like–unlike) interactions.

Molecules such as these with two secondary amide functions are preferable to the corresponding non-methylated derivatives (I) as biological models,[28] and in retrospect it would have been better to have investigated the present systems initially. This was considered at the time, but we were concerned that the addition of the *N'*-methyl function would have a deleterious effect on the solubilities of the compounds and consequently limit the experimental information which could be obtained. Our current experience indicates that incorporation of a *N'*-methyl function has little effect upon solubility.

EXPERIMENTAL

METHODS

Enthalpies of dilution were determined using a LKB batch microcalorimetric system.[1] The calorimeter was modified so that the signal from the thermopiles was, after amplification, digitised and then integrated using a microcomputer. The isopiestic procedure and apparatus have been described previously.[29]

PREPARATION AND PURIFICATION OF MATERIALS

Pure amino acids were a gift from Roche Products Ltd (Welwyn Garden City) or purchased; glycine (A.R. grade) was from Fisons; L-alanine and L-leucine were from Aldrich. The terminally blocked monomers were synthesised from the *N*-benzyloxycarbonyl-*N'*-methylamide of the amino acids, and were obtained both optically and chemically pure. *N*-Benzyloxycarbonyl-*Z*-amino acids were prepared following the method of Bergmann and Zervas.[30] *Z*-Amino acid methylamides were prepared according to the method described for the L-alanine derivative, following the method of Anderson and Zimmerman.[31] Tables 1 and 2 summarise the physical constants and p.m.r. data on the substances and their precursors.

Table 1. Summary of physical data on substances synthesised

compound	crystallisation solvents	yield (%)	m.p./°C	$[\alpha]_D^{21}$	ref.	t.l.c.[a]	found (%) (calculated) C	H	N
Z-Gly-OH	EtOAc + petrol	88	119–20	—	32	0.45 (A)	—	—	—
Z-L-Ala-OH	EtOAc + petrol	81	85–6	−15.5 (*c*2, HOAc)	31	0.91 (B)	—	—	—
Z-L-Leu-OH	oil	98	—	—	33	0.73 (B)	—	—	—
Z-Gly-NHMe	EtOH + Et_2O	80	109–8	—	34	0.43 (C)	59.30 (59.45)	6.60 (6.35)	12.80 (12.60)
Z-L-Ala-NHMe	EtOAc	73	128–9	−9.0 (*c*1, EtOH)		0.52 (C)	61.25 (61.00)	6.85 (6.85)	11.60 (11.85)
Z-L-Leu-NHMe	EtOH + Et_2O	68	127–8	−16.0 (*c*3, EtOH)		0.71 (C)	64.75 (64.75)	7.80 (7.95)	10.15 (10.05)
Ac-Gly-NHMe	EtOH + Et_2O	72	156–7.5	—	35	0.68 (D)	46.40 (46.15)	8.00 (7.75)	21.40 (21.50)
Ac-L-Ala-NHMe	EtOH + Et_2O	76	182–3	−58.0 (*c*1, EtOH)	35, 36	0.41 (E)	50.30 (50.00)	8.50 (8.40)	19.70 (19.45)
Ac-L-Leu-NHMe	EtOH + hexane	73	165–6	−44.0 (*c*1, EtOH)	35, 36	0.55 (E)	58.25 (58.05)	9.70 (9.75)	15.20 (15.05)
Z-Gly-L-Ala-NH_2	EtOH + Et_2O	76	182–3	−16.5 (*c*0.4, MeOH)	37	0.27 (F)	56.00 (55.90)	5.95 (6.15)	14.80 (15.05)
Ac-Gly-L-Ala-NH_2	H_2O	98	263–4	−35.5 (*c*0.3, H_2O)	38	0.24 (B)	44.70 (44.90)	6.80 (7.00)	22.75 (22.45)

[a] T.l.c. systems as follows: (A) EtOAc:pyridine:AcOH:H_2O, 240:20:6:11; (B) n-butyl alcohol:AcOH:H_2O, 4:1:1; (C) chloroform:methanol, 19:1; (D) chloroform:methanol:AcOH:H_2O, 60:18:1:1.5; (E) chloroform:methanol:AcOH:H_2O, 120:18:1:1.5; (F) chloroform:methanol, 9:1.

Table 2. Proton chemical-shift data[a] for substances synthesised

compound (solvent)	C_6H_5	$ArCH_2$	$C_1^{\alpha}H_n$	$C_2^{\alpha}H_n$	$C^{\beta}H_n$	$C^{\gamma}H$	$C^{\delta}H_3$	NCH_3	CH_3CO		amide protons[b]		
Z-Gly-NHMe	7.33	5.09	3.81	—	—	—	—	2.77	—	6.55–6.30	5.81	—	—
	5H	2H	2H	—	—	—	—	3H	—	1H br	1H br	—	—
($CDCl_3$)	s	s	d *J* 6-Hz	—	—	—	—	d *J* 5-Hz	—	m	m	—	—
Z-L-Ala-NHMe	7.33	5.08	4.32–4.14	—	1.36	—	—	2.76	—	6.55–6.31	5.61	—	—
	5H	2H	1H	—	3H	—	—	3H	—	1H br	1H	—	—
($CDCl_3$)	s	s	m	—	d *J* 7-Hz	—	—	d *J* 5-Hz	—	m	d *J* 7-Hz	—	—
Z-L-Leu-NHMe	7.33	5.10, 5.00	4.31–4.15	—	1.57–1.45	1.75–1.45	0.91	2.73	—	6.78	5.81	—	—
	5H	2H	1H br	—	2H	1H	6H	3H	—	1H br	1H br	—	—
($CDCl_3$)	s	dd *J* 12-Hz	m	—	m	m	d *J* 5-Hz	d *J* 5-Hz	—	s	d *J* 9-Hz	—	—
Ac-Gly-NHMe	—	—	3.91	—	—	—	—	2.82	2.05	6.75	6.61	—	—
	—	—	2H	—	—	—	—	3H	3H	1H br	1H br	—	—
($CDCl_3$)	—	—	d *J* 6-Hz	—	—	—	—	d *J* 5-Hz	s	s	s	—	—
Ac-L-Ala-NHMe	—	—	5.88	—	1.37	—	—	2.77	1.98	7.30	—	—	—
	—	—	1H	—	3H	—	—	3H	3H	2H br	—	—	—
($CDCl_3$)	—	—	dq(ol) *J* 6-Hz	—	d *J* 7-Hz	—	—	d *J* 5-Hz	s	m	—	—	—
Ac-L-Leu-NHMe	—	—	4.65–4.50	—	1.65	1.65	0.96, 0.93	2.76	1.99	7.60–7.39	—	—	—
	—	—	1H	—	2H	1H	3H, 3H	3H	3H	2H br	—	—	—
($CDCl_3$)	—	—	m	—	m	m	2d *J* 5-Hz	d *J* 5-Hz	s	m	—	—	—
Z-Gly-L-Ala-NH_2	7.32	5.15	3.58	4.28	1.26	—	—	—	—	7.89	7.31–7.27	6.96	—
	5H	2H	2H	1H	3H	—	—	—	—	1H	2H br	1H br	—
([$^2H_6$]DMSO)	s	s	d *J* 7-Hz	m	d *J* 7-Hz	—	—	—	—	d *J* 7-Hz	s	s	—
Ac-Gly-L-Ala-NH_2	—	—	3.70	4.22	1.25	—	—	—	1.89	8.19	8.02	7.33	6.99
	—	—	2H br	1H br	3H	—	—	—	3H	1H br	1H br	1H br	1H br
([$^2H_6$]DMSO)	—	—	m	m	d *J* 7-Hz	—	—	—	s	s	s	s	s

[a] Labelling nomenclature for peptides was as follows. Carbon atoms in an amino acid residue have been labelled α, β, γ *etc.* from the carbonyl carbon atom, and amino acid residues have been numbered from the *N*-terminus, following standard convention.[39] C_2^{β} is thus the β-carbon atom of the second amino acid residue along the peptide from the *N*-terminus. [b] These are not assigned.

N-BENZYLOXYCARBONYL-*N'*-METHYL-L-ALANINAMIDE

Z-L-Alanine (11.2 g, 0.05 mol) and *N*-ethylmorpholine (NEM) (6.3 cm^3. 0.05 mol) were dissolved in dry tetrahydrofuran (THF) (100 cm^3). The mixture was cooled to −15 °C by means of an ethandiol + liquid-nitrogen slush bath. Isobutyl chloroformate (Bu^iCF) (6.6 cm^3, 0.05 mol) was added and the mixture was stirred vigorously at −15 °C for 5 min. Then an ethanolic solution of methylamine (33%) (13.3 cm^3, 0.1 mol, 2.0 equiv.) was introduced cautiously and stirring was continued for 20 min while the temperature was allowed to rise slowly to ambience. Solvent was removed *in vacuo* (the bath temperature was not allowed to exceed 30 °C) and the residual oily solid was partitioned between ethyl acetate (3 × 150 cm^3) and water (150 cm^3). The combined organic phase was washed sequentially with 10% (w/v) aqueous citric acid, 10% (w/v) aqueous sodium hydrogen carbonate and twice with aqueous saturated sodium chloride (washed neutral). The organic phase was then dried over anhydrous magnesium sulphate, filtered and evaporated *in vacuo*. The resulting colourless, crystalline solid was recrystallised from ethyl acetate.

N-ACETYL-*N'*-METHYL-L-ALANINAMIDE

Z-*N'*-Methyl-L-alaninamide (11.8 g, 0.05 mol) was dissolved in 80% (v/v) aqueous acetic acid (200 cm^3) and the solution was purged thoroughly with nitrogen. The compound was hydrogenolysed overnight at room temperature and at a slight positive pressure, using 5% Pd/C catalyst (0.5 g). After filtration under nitrogen, the hydrogenolysate was added to a precooled mixture of acetic anhydride (5.2 cm^3, 0.055 mol, 1.1 equiv.) and pyridine (200 cm^3) at −15 °C. The mixture was stirred at this temperature for 15 min and then evaporated to dryness *in vacuo* at low temperature. Final traces of pyridine and water were removed by lyophyllisation from toluene and then from dry ethanol. The resulting colourless crystalline solid was then recrystallised from EtOAc + diethyl ether and subsequently EtOH + Et_2O mixtures to purity.

N-ACETYL-*N'*-METHYL-L-LEUCINAMIDE

The title compound was prepared in an analogous fashion to *N*-acetyl-*N'*-methyl-L-alaninamide; however, purification was executed by elution from Kieselgel 60H (Merck) with a mixture of chloroform, methanol, acetic acid and water in the ratio 120:18:1:15 and recrystallisation from a EtOH + hexane mixture.

N-ACETYL-*N'*-METHYLGLYCINAMIDE

Z-*N'*-Methylglycinamide (16.7 g, 0.075 mol) was hydrogenolysed overnight in a solution of *p*-toluenesulphonic acid monohydrate (14.3 g, 0.075 mol) and MeOH (400 cm^3). After filtration, the hydrogenolysate was evaporated to dryness *in vacuo* at low temperature and re-evaporated from EtOAc to remove traces of MeOH. The oily residual dipeptide *p*-toluenesulphonate salt was acetylated as described for *N*-acetyl-*N'*-methyl-L-alaninamide. After removal of solvent the resulting mixture of crude product and pyridinium *p*-toluenesulphonate was dissolved in water (250 cm^3), cooled in an ice bath and stirred with an excess of Amberlite MB-3 ion-exchange resin for 30 min. After filtration and evaporation *in vacuo* the product was dried by re-evaporating from a dry 1:1 EtOAc + EtOH mixture. The product, a colourless crystalline solid, was recrystallised from an EtOH + Et_2O mixture to purity.

N-BENZYLOXYCARBONYLGLYCYL-L-ALANINAMIDE

Z-L-Alaninamide was synthesised from *Z*-L-alanine in a manner analogous to the synthesis of the methylamide, substituting the ethanolic methylamine solution by 0.880 aqueous ammonia; (65%) m.p. 131–132 °C (*ex* EtOAc) [lit.[37] m.p. 133 °C (*ex* EtOH + hexane)]. The *p*-toluenesulphonate salt of L-alaninamide was obtained by hydrogenolysis in a solution of 1 equivalent of *p*-toluenesulphonic acid monohydrate in dimethylformamide (DMF). *Z*-Glycine (9.6 *g*, 0.046 mol) and NEM (5.8 cm^3, 0.046 mol) were dissolved in dry DMF (200 cm) and the mixture was cooled with stirring to −15 °C. Bu^iCF (6.0 cm^3, 0.046 mol) was added, and after 5 min stirring a solution of the *p*-toluenesulphonate salt of L-alaninamide (0.046 mol) and NEM (5.8 cm^3, 0.046 mol) in DMF (500 cm^3) was introduced slowly. After a further 15 min stirring at −15 °C the solvent was removed *in vacuo* at a low temperature, and the residual oily solid

was partitioned between dichloromethane (3×250 cm^3) and water (150 cm^3). The combined organic extracts were washed and dried as described for *Z*-*N'*-methyl-L-alaninamide. The colourless microcrystalline product was recrystallised from an EtOH + Et_2O mixture.

N-ACETYLGLYCYL-L-ALANINAMIDE

Z-Glycyl-L-alaninamide (10.1 g, 0.036 mol) was dissolved in a 1:1 mixture of 80% aqueous HOAc and DMF (400 cm^3) and hydrogenolysed overnight. Acetylation and purification were carried out as described for *N*-acetyl-*N'*-methyl-L-alaninamide. The colourless crystalline product was recrystallised from an EtOH + Et_2O mixture to purity.

RESULTS

The thermodynamic formalism used for treating solutions containing non-electrolytic solutes has been given elsewhere.[1,2] The excess Gibbs free energy (G^{ex}) of a solution containing 1 kg of solvent and two solutes A and B is represented by

$$G^{ex} = g_{AA} m_A^2 + g_{BB} m_B^2 + 2g_{AB} m_A m_B + \text{higher-order terms} \quad (1)$$

in which g_{ij} denotes the free-energy interaction coefficient of the subscripted species and m_i is the molality of species i. Eqn (1) can be transformed to give[2] the following expressions for the osmotic coefficients (ϕ) of solutions containing only solute A, only solute B and solutes A and B:

$$\phi_A = 1 + (g_{AA} m_A + 2g_{AAA} m_A^2 + \ldots)/\boldsymbol{RT} \quad (2)$$

$$\phi_B = 1 + (g_{BB} m_B + 2g_{BBB} m_B^2 + \ldots)/\boldsymbol{RT} \quad (3)$$

$$\begin{aligned}\phi_{AB} &= 1 + (m/\boldsymbol{RT})[(g_{AA} y_A^2 + g_{BB} y_B^2 + 2g_{AA} y_A y_B) \\ &\quad + 2m(g_{AAA} y_A^3 + g_{BBB} y_B^3 + 3g_{AAB} y_A^2 y_B + 3g_{ABB} y_A y_B^2) \\ &\quad + \ldots] \\ &= 1 + (g_2 + 2mg_3 + \ldots) m/\boldsymbol{RT}. \end{aligned} \quad (4)$$

In eqn (3) and (4) m is the osmolality ($m_A + m_B$) and y_A and y_B are the solute mole fractions of A and B, respectively. Consequently the homotactic interaction coefficients can be obtained from eqn (2) and (3) and the heterotactic interaction coefficients from eqn (4) using known homotactic coefficients.

The experimental results obtained from the isopiestic experiments are presented in table 3. In all of the systems investigated aqueous urea solutions were used as the references. The osmotic coefficients given in this table were obtained using the data of Ellerton and Dunlop.[25] The data set for each system was fitted to a polynomial in solute molality,[1,2] and the resulting interaction coefficients are given in table 4.

The corresponding expression to eqn (1) for the excess enthalpy per kg of solvent (H^{ex}) is

$$H^{ex} = h_{AA} m_A^2 + h_{BB} m_B^2 + 2h_{AB} m_A m_B + \ldots \quad (5)$$

where h_{ij} is the enthalpic analogue of g_{ij}. This leads to the following expressions for the excess enthalpies of solutions containing one and two solvents:

$$H_A^{ex} = m_A^2 (h_{AA} + h_{AA} m_A + \ldots) \quad (6)$$

$$H_B^{ex} = m_B^2 (h_{BB} + h_{BBB} m_B + \ldots) \quad (7)$$

$$\begin{aligned} H_{AB}^{ex} &= m^2[(h_{AA} y_A^2 + h_{BB} y_B^2 + 2h_{AB} y_A y_B) \\ &\quad + m(h_{AAA} y_A^3 + h_{BBB} y_B^3 + 3h_{AAB} y_A^2 y_B + 3h_{ABB} y_A y_B^2) \\ &\quad + \ldots]. \end{aligned} \quad (8)$$

Table 3. Isopiestic molalities obtained at 25 °C for the systems investigated

		m/mol kg^{-1}					
m(urea) /mol kg^{-1}	ϕ(urea)[a]	GMe	AMe	LMe	GMe+AMe $y = 0.4983$[b]	GMe+LMe $y = 0.5016$[b]	AMe+LMe $y = 0.5015$[b]
0.4324	0.9822	0.4277	—	—	0.4283	0.4468	0.4528
0.4609	0.9811	0.4571	—	—	0.4566	0.4799	0.4861
0.5041	0.9795	0.4984	—	—	0.4985	0.5292	0.5340
0.5462	0.9779	0.5383	—	—	0.5392	0.5783	0.5827
0.6113	0.9755	0.6009	—	—	0.6019	0.6467	0.6511
0.6606	0.9737	0.6466	—	—	0.6493	0.7024	0.7068
0.3305	0.9862	—	0.3308	—			
0.3529	0.9853	—	0.3538	0.3858			
0.3751	0.9845	—	0.3757	0.4191			
0.3986	0.9835	—	0.3998	0.4494			
0.4850	0.9802	—	0.4860	0.5499			
0.5174	0.9791	—	—	0.6022			
0.7203	0.9716	—	0.7158	0.8803			
0.7862	0.9692	—	0.7825	0.9958			
0.8506	0.9670	—	0.8445	1.0853			

[a] Taken from ref. (40). [b] y is the solute mole fraction of the first-mentioned component.

Table 4. Excess Gibbs free energy of interaction parameters at 25 °C for the N'-methylamide systems investigated

solute species					
A	B	g_2[a] /J kg mol^{-2}	g_3[a] /J kg^2 mol^{-3}	$\sigma/10^3$ /mol kg^{-1}[b]	y[c]
GMe	GMe	−76.8 (26.2)[c]	66.2 (38.4)	0.6	—
GMe	AMe	−62.9 (7.0)	32.7 (10.2)	0.2	0.4983
GMe	LMe	−303.1 (15.3)	—	2.0	0.5016
AMe	AMe	−119.4 (20.2)	31.4 (16.6)	1.0	—
AMe	LMe	−389.9 (48.2)	74.4 (59.4)	1.4	0.5015
LMe	LMe	−680.8 (56.9)	53.8 (28.5)	4.6	—

[a] The number in parentheses represents the 95% confidence range of the coefficient. [b] σ is the standard error of the least-squares fit. [c] In the ternary systems y is the solute mole fraction of solute A.

The enthalpy-of-interaction coefficients were obtained as before,[12] by the use of dilution experiments. The experimental enthalpy change (q) arising from a dilution is given by[12]

$$q = n(m' - m)[h_2 + (m' + m)h_3 + \ldots] \quad (9)$$

in which n is the number of moles of solute species used in the dilution experiment and m' and m are the osmolalities after and before dilution. We can identify h_2 with h_{AA} or h_{BB} for one-component solute solutions and with the first term in parentheses on the right-hand side of eqn (8) for two-component solute solutions. The higher-order

Table 5. Experimental enthalpies of dilution at 25 °C

m/mol kg^{-1}	$n/10^{-3}$ mol	m'/mol kg^{-1}	$-q$/J	$\Delta/10^{-4}$ J
		N-acetyl-*N*′-methylglycinamide		
0.4127	0.8484	0.2048	0.1005	26
0.4127	0.8348	0.1386	0.1334	3
0.4127	1.2291	0.2834	0.0920	9
0.4127	0.2923	0.0611	0.0580	21
0.8553	1.7102	0.4194	0.4283	74
0.8553	1.6392	0.2714	0.5633	−39
0.8553	3.2205	0.5536	0.5706	−29
		N-acetyl-*N*′-methyl-L-alaninamide		
0.8040	1.0949	0.3872	0.5243	145
0.8040	1.4838	0.2534	0.9661	−15
0.8040	0.7403	0.1510	0.5703	5
0.8040	0.6295	0.1360	0.5080	−114
0.3970	0.7868	0.1961	0.1889	−22
0.3970	1.1427	0.2592	0.1880	−21
0.3970	0.5732	0.1277	0.1836	−13
		N-acetyl-*N*′-methyl-L-leucinamide		
0.7423	1.2997	0.3494	1.7472	−8
0.7423	1.9411	0.4725	1.8025	−114
0.7423	0.7082	0.1455	1.4346	108
0.7423	2.6367	0.5774	1.4866	8
0.3510	0.6860	0.1691	0.4241	26
0.3510	0.6825	0.1138	0.5467	69
		N-acetyl-*N*′-methylglycinamide + *N*-acetyl-*N*′-methyl-L-alaninamide		
		$y = 0.4954$[a]		
0.3786	0.7626	0.1858	0.1309	49
0.3786	0.7451	0.1247	0.1750	−3
0.3786	1.1338	0.2473	0.1366	8
0.3786	0.3936	0.0769	0.1144	−48
0.3786	0.4923	0.1142	0.1218	−16
		N-acetyl-*N*′-methylglycinamide + *N*-acetyl-*N*′-methyl-L-leucinamide		
		$y = 0.5095$[a]		
0.4268	0.7341	0.1995	0.2805	−18
0.4268	0.8540	0.1406	0.4016	67
0.4268	0.3787	0.0991	0.2050	23
0.4268	0.4148	0.0836	0.2353	25
0.4268	0.5419	0.1043	0.3032	−113
		N-acetyl-*N*′-methyl-L-alaninamide + *N*-acetyl-*N*′-methyl-L-leucinamide		
		$y = 0.4900$[a]		
0.2812	0.5587	0.1391	0.1633	1
0.2812	0.4934	0.0865	0.1988	2
0.2812	0.8007	0.1826	0.1616	−1
0.2812	0.3147	0.0605	0.1444	−1
0.2812	0.4730	0.0918	0.1856	−2
		N-acetylglycyl-L-alaninamide[b]		
0.0711	0.2776	0.0463	0.0009	4
0.0634	0.1252	0.0207	0.0007	3
0.0634	0.2343	0.0421	0.0012	−3
0.0647	0.1315	0.0216	0.0013	−2
0.0647	0.2340	0.0412	0.0014	−4

[a] y is the solute mole fraction of the first-mentioned component. [b] The data obtained for this system are of relatively low quality because of the restricted molality range investigated. The low solubility of the substance precluded a more extensive investigation.

Table 6. Parameter of the excess enthalpy of interaction for the peptide systems investigated

solute species[a]					
A	B	h_2/J kg mol^{-2}	h_3/J kg^2 mol^{-3}	$\sigma/10^{-3}$ J mol^{-1} [b]	y[c]
GMe	GMe	584.5 (5.8)[d]	—	3.9	—
GMe	AMe	923.3 (29.6)	—	3.5	0.4954
GMe	LMe	1670.2 (48.9)	—	6.9	0.5095
AMe	AMe	1180.8 (15.9)	—	7.7	—
AMe	LMe	2166.7 (28.4)	−259.2 (72.0)	0.2	0.4900
LMe	LMe	3420.1 (20.8)	—	7.8	—
GA[a]	GA	189.9 (78.0)	—	0.4	—

[a] The abbreviations used for the compunds are given in the footnote to table 9. [b] is the standard error of the least-squares fit. [c] In the ternary systems y is the solute mole fraction of solute A. [d] The number in parentheses represents the 95% confidence limit of the coefficient.

Table 7. Free-energy, enthalpy and entropy pairwise interaction coefficients at 25 °C for the *N'*-methylamide peptide systems investigated

solute species				
A	B	g_{AB}[a]/J kg mol^{-2}	h_{AB}[a]/J kg mol^{-2}	Ts_{AB}[a]/J kg mol^{-2}
GMe	GMe	−76.8 (26.2)	584.5 (5.8)	661.3 (32.0)
GMe	AMe	−27.6 (37.2)	958.6 (70.2)	986.2 (107.4)
GMe	LMe	−229.3 (72.2)	1391.5 (110.9)	1620.8 (183.1)
AMe	AMe	−119.4 (20.2)	1180.8 (15.9)	1300.2 (36.1)
AMe	LMe	−380.4 (135.0)	1988.1 (75.3)	2369.5 (210.3)
LMe	LMe	−680.8 (56.9)	3420.1 (20.8)	4100.9 (77.7)

[a] The number in parentheses represents the 95% confidence limit of the coefficient.

terms in eqn (9) are for binary systems; they are composite functions of several terms and will not be considered further.

The primary experimental data obtained from the dilution experiments are presented in table 5, and in table 6 we give the coefficients, obtained from a least-squares analysis, of the several systems studied. The various pairwise interaction coefficients are collected in table 7. Included in this table are the entropy coefficients calculated from the equation

$$Ts_{ij} = (h_{ij} - g_{ij}).$$

DISCUSSION

It is instructive, before entering into a detailed discussion of the results obtained, to make some qualitative comparisons of the intermolecular interaction parameters for the peptides containing a primary amide function with those containing secondary amide functions.

In fig. 1 we present a graphical summary of the interaction parameters, and it is clear

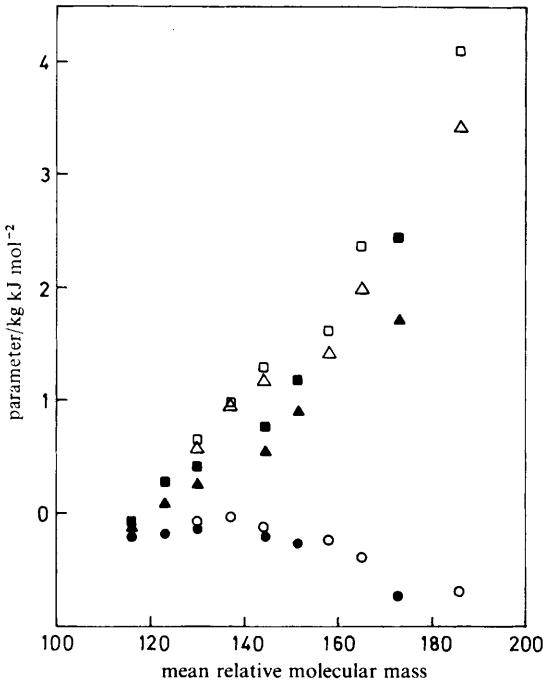


Fig. 1. Plot of interaction parameters against mean relative molecular mass. The closed symbols refer to C-terminal amides (●, g_{AB}; ▲, h_{AB}; ■, Ts_{AB}) and the open symbols refer to the methylamides (○, g_{AB}; △, h_{AB}; □, Ts_{AB}).

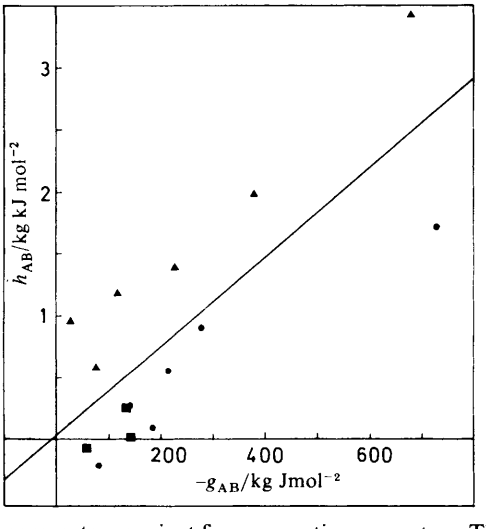


Fig. 2. Plot of enthalpic parameters against free-energetic parameters. The data for the systems are given in tables 9 and 10. ■, Simple amides; ●, C-terminal amides; ▲, methylamides.

Table 8. Group parameters for amide and peptide systems (units are J kg mol^{-2})

		j	
i		CH_2	Pep
CH_2	G_{ij}	−19.6 (20.8)[a]	—
	H_{ij}	25.0 (12.9)	—
Pep	G_{ij}	22.1 (53.4)	−50.5 (137.8)
	H_{ij}	80.5 (29.1)	−291.6 (59.1)

[a] The parenthetical term is the 95% confidence limit.

from this that in all cases investigated the introduction of the N'-methyl group has little or no effect on the interactions as monitored by the free-energy terms. However, there are marked changes in the enthalpic terms; in the most extreme examples studied (the leucyl derivatives), methylation of the terminal primary-amide function leads to an increase in the enthalpic term by almost a factor of two. These two observations indicate the interplay between entropic and enthalpic effects in determining the nett free-energetic effects. This is illustrated in a different way in fig. 2, where we present the enthalpic terms plotted against the free-energetic term. This shows that for the N'-methylated compounds the entropic contribution (Ts_{AB}) is *ca.* 1000 J kg mol^{-2} more positive than that for the corresponding primary amides.

One of the major objectives of the present series of studies, as mentioned earlier, is to try to discover rules which allow predictions of non-bonding interactions in aqueous solutions. One such method which has attracted considerable attention is the group-additivity[9] approach. In essence this procedure assumes that each group on solute species A interacts with each group on solute species B. The nett interaction between the two species is then given by an expression of the form

$$x_{AB} = X_{ij}\, n_i^A\, n_j^B \tag{10}$$

where x_{AB} is a thermodynamic interaction coefficient representing pairwise interactions between solutes A and B, n_i^A and n_j^B denote the numbers of groups of type i on species A and type j on species B. X_{ij} is the intensive factor representing the interaction of one group of type i with one group of type j.

In the earlier works,[1-19] in order to reduce the number of X_{ij} parameters, groups of similar structural and chemical types were interrelated by numerical factors. For example, when considering molecules containing methyl, methylene and methine groups, the methylene group was taken as the primary unit and the methyl and methine groups were taken to be equivalent to 1.5 and 0.5 primary units, respectively.[7, 41] This approximation is simply a convenience when relatively limited data sets are considered, and it is not a necessary assumption for the application of the group-additivity approach. In terms of this approximation, and also assuming (as before) that the two groups —CONH— and —$CONH_2$ are equivalent, for the systems investigated here the thermodynamic coefficients for free energy and enthalpy are given by

$$g_{AB} = n^A_{CH_2} n^B_{CH_2} G_{CH_2\text{-}CH_2} + (n^A_{Pep} n^B_{CH_2} + n^B_{Pep} n^A_{CH_2}) G_{CH_2\text{-}Pep} + n^A_{Pep} n^A_{Pep} G_{Pep\text{-}Pep} \tag{11}$$

$$h_{AB} = n^A_{CH_2} n^B_{CH_2} H_{CH_2\text{-}CH_2} + (n^A_{Pep} n^B_{CH_2} + n^B_{Pep} n^A_{CH_2}) H_{CH_2\text{-}Pep} + n^A_{Pep} n^B_{Pep} H_{Pep\text{-}Pep}. \tag{12}$$

Table 9. Experimental and calculated values for h_{AB} for C-terminal *N'*-methylamides, amides and some simple amides.

solute species[a]		h_{AB} (exptl) /J kg mol^{-2}	h_{AB} (calc) /J kg mol^{-2}
A	B		
G	G	−220	−205
A	A	268	267
V	V	1260	1361
L	L	1714	1983
G	A	86	18
G	V	385	465
G	L	549	689
A	V	591	764
A	L	899	1013
V	L	1486	1660
GMe	GMe	585	522
AMe	AMe	1181	1069
LMe	LMe	3420	3010
GMe	AMe	959	783
GMe	LMe	1392	1556
AMe	LMe	1998	1927
NMF	NMF	292	130
NMA	NMA	234	416
NMP	NMP	636	953
NMA	NBA	1498	1575
NMF	NBA	883	653
NMF	NMP	540	391
NMF	NMA	368	261
NMA	NBA	628	883
NMA	NMP	394	572
NMP	NBA	1080	1114
GG	GG	−646	−628
AG	AG	284	55
GA	GA	190	55
AA	AA	939	788
GG	G	−211	−364
AA	A	488	462
GGG	GGG	−1499	−1262
GGG	G	−544	−522
FOR	FOR	−115	−205
ACE	ACE	12	6
PRP	PRP	249	267

[a] The abbreviations used are as follows G, *N*-acetylglycinamide; A, *N*-acetyl-L-alaninamide; V, *N*-acetyl-L-valinamide; L, *N*-acetyl-L-leucinamide; GMe, *N*-acetyl-*N'*-methylglycinamide; AMe, *N*-acetyl-*N'*-methyl-L-alaninamide; LMe, *N*-acetyl-*N'*-methyl-L-leucinamide; NMF, *N*-methylformamide; NMA, *N*-methylacetamide; NMP, *N*-methylpropionamide; NBA, *N*-butylacetamide; GG, *N*-acetylglycylglycinamide; AG, *N*-acetyl-L-alanylglycinamide; GA, *N*-acetyl-glycyl-L-alaninamide; AA, *N*-acetyl-L-alanyl-L-alaninamide; GGG, *N*-acetyl-glycylglycylglycinamide; FOR, formamide; ACE, acetamide; PRP, propionamide.

Table 10. Experimental and calculated values for g_{AB} for monomeric systems containing primary and secondary amide functions

solute species[a]		$-g_{AB}$ (exptl) /kg J mol^{-2}	$-g_{AB}$ (calc) /kg J mol^{-2}
A	B		
G	G	83[b]	104
A	A	144[b]	133
L	L	732[b]	457
G	A	186[b]	109
G	L	217[b]	124
A	L	280[b]	207
GMe	GMe	77	163
AMe	AMe	119	251
LMe	LMe	681	752
GMe	AMe	28	197
GMe	LMe	229	300
AMe	LMe	381	413
FOR	FOR	60[c]	33
ACE	ACE	145[c]	28
PRP	PRP	135[c]	63

[a] Values taken from ref. (27). [b] See footnote to table 9 for the abbreviations used. [c] Values taken from ref. (1).

We have used eqn (11) and (12) to analyse the results obtained in the present investigation along with those obtained from some earlier studies on amide- and peptide-containing systems. The group-interaction parameters for the free-energy and enthalpy coefficients are given in table 8. Tables 9 and 10 give the observed and fitted values of the coefficients for the systems used in the analyses.

The numerical values of the group-interaction enthalpy parameters differ from the values obtained[1-3] using more limited data sets but are in close agreement with those obtained recently by Tasker and Wood.[11] There are also some changes in the free-energy group parameters; however, given the still very limited data set and the uncertainties in the parameters, it would be unwise to draw any conclusions from these differences. It is not our intention to discuss here the derived group parameters in detail, since we are involved in investigations of systems containing other amino acid residues. These include phenylalanyl- and prolyl-containing peptides, and a comprehensive discussion will be given shortly. However, the $G_{CH_2-CH_2}$ term and the $G_{Pep-Pep}$ term are both negative and indicate attractive interactions between the subscripted groups, whereas the G_{CH_2-Pep} term is positive and thus indicates repulsion between the CH_2 and Pep groups. It also seems that the molecular differences between CH_2–CH_2 and Pep–Pep interactions are highlighted by the difference in the signs of their group-enthalpy parameters.

In the spirit of our search for empirical rules to represent the interactive energetics of a large number of systems using the minimum number of parameters, we have explored[28] several variations on the group-additivity approach. For example, we have treated the results where, rather than using the numbers of equivalent CH_2 and peptide groups, we have normalised the groups using surface areas (calculated from molecular models[42]). No significant differences in the quality of the data fittings were obtained

using this modification. We have also pursued[28] an analysis based on the McMillan–Mayer[43, 44] theory and transpositions,[45] since it is known that such an approach should be more closely related to molecular events than approaches based on Lewis–Randall[45] coefficients. The qualities of the data fittings obtained were marginally worse for the enthalpy coefficients and better for the free-energy coefficients than those obtained from the other analyses. The qualitative conclusions drawn above were confirmed.

To summarise, it appears that of the several representational approaches we explored none offered an advantageous improvement over the usual analysis[1–19] with regard to how well they represented experimental data. Moreover, it seems clear that one cannot expect too much from a group-additivity approach. Notwithstanding this, such approaches certainly can be used to represent a large body of experimental data in a fairly compact way and frequently have remarkably good predictive utility. However, there exists an increasing number of examples where group-additivity schemes fail rather badly.[12, 13, 28, 46] A major deficiency in the possible application of such schemes to complex molecules is that no provision is made for the effect of intramolecular interactions upon intermolecular associations. This, and some other aspects of the general problems of rationalising the behaviour of oligopeptides in aqueous solutions, will be discussed in some later papers.

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