Journal of Solution Chemistry, Vol. 13, No. 11, 1984

Aqueous Solutions Containing Amino Acids and Peptides. Part 19: The Enthalpic Coefficients for the Interactions of N-Acetylsarcosinamide with 2-(N-acetylamino)acyl Amides at 25 °C

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Received May 31, 1984

Enthalpies of dilution both of solutions of N-acetylsarcosinamide and of ternary solutions equimolal in N-acetylsarcosinamide and N-acetylglycinamide, N-acetyl-L-alaninamide, N-acetyl-L-valinamide or N-acetyl-L-leucinamide have been determined by a microcalorimetric method. The results were employed to calculate the pairwise enthalpic coefficients for both homotactic (like-like) and heterotactic (like-unlike) solute interactions. These pairwise interaction coefficients have been analyzed by means of a group additivity approach and some comments on the utility of this, when applied to such systems, are made.

KEY WORDS: Group additivity; amino acids; aqueous solutions; enthalpy of dilution.

1. INTRODUCTION

This study is part of a continuing series[1-5] of investigations performed in this laboratory concerning the interactions which occur between terminally protected amino acids and peptides in aqueous solutions. The aims and motivations of this work have been summarized earlier.[1-4]

Previously the interactions occurring in aqueous solutions of some N-acetyl amides, N-acetyl methylamides, and N-acetyl peptide

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0095-9782/84/1100-0789$03.50/0

amides of the α-amino acids glycine, L-alanine, L-valine, L-leucine and L-phenylalanine have been determined and assessed.[1-5] The residues present in these compounds are representative of the class, occurring naturally in proteins, which are termed apolar or hydrophobic amino acids.

Proline is also classed as an apolar amino acid and is widely recognized to have especial importance in determining the properties of both small and large peptides. However, it differs from the other apolar species in two important respects. Firstly, it is an α-imino acid, and secondly, its geometry is constrained by the constriction of the side-chain into a five-membered pyrolidine ring.

The smallest and structurally the simplest α-imino acid is sarcosine, N-methylglycine. We have studied the interactions of a derivative of this in order to explore the consequences of incorporating a tertiary amide bond into a monomeric peptide molecule. Investigations have been made on the homotactic behavior of N-acetylsarcosinamide (SAR) and the heterotactic interactions of SAR with N-acetylglycinamide (GLY), N-acetyl-L-alaninamide (ALA), N-acetyl-L-valinamide (VAL) and N-acetyl-L-leucinamide (LEU) by enthalpy of dilution measurements. The interactions of the sarcosyl-residue will be of comparative use in subsequent considerations of the prolyl residue.

2. EXPERIMENTAL

2.1. Apparatus and Methods

The microcalorimeter used and its ancillary equipment have been described previously.[1,4] ^{1}H NMR spectra were recorded using a JEOL 220 MHz instrument at ambient temperature with TMS as internal reference.

2.2. Preparation and Purification of Materials

N-Phenylmethoxycarbonylsarcosine. Sarcosine (Aldrich Chemical Co.) (44.5 g, 0.5*m*) was dissolved in aqueous sodium hydroxide (2*M*, 250 ml) and the solution cooled below 5 °C. Phenylmethylchloroformate (85 ml, 0.6*m*) and aqueous sodium hydroxide (2*M*, 250 ml) were added synchronously from separate dropping funnels over a period of 30 min with vigorous mechanical stirring. The cold mixture was stirred for 2 h and then allowed to warm to room temperature. The resulting viscous liquid was washed twice with diethyl ether (250 ml). The

aqueous phase was poured onto ice in a beaker and rapidly acidified to pH 1-2 with aqueous hydrochloric acid (6*M*), with external cooling. The product separated as an oil and was rapidly extracted with ethyl acetate (3 × 250 ml). The combined organic extract was washed with distilled water and with saturated aqueous sodium chloride, dried over anhydrous $MgSO_4$, filtered and evaporated *in vacuo*. The resulting oil crystallized after standing for a protracted period over P_2O_5 *in vacuo*; yield 91.1g, 82%; *m.p.* 65-6 °C; [lit.[6,7] *m.p.* 53-4 °C]. R_f 0.62 (*n*-butanol: acetic acid: water, 4:1:1). δ ($CDCl_3$) 10.39 (1H, *s*, COO*H*), 7.35 (1.67H, *s*, C_6H_5), 7.31 (3.33H, *s*, C_6H_5), 5.17 (1.33H, *s*, ArCH_2), 5.15 (0.67, *s*, ArCH_2), 4.11 (1.33H, *s*, CH_2), 4.04 (0.67H, *s*, CH_2), 4.11 (1.33H, *s*, CH_2), 4.04 (0.67H, *s*, CH_2), 2.99 (3H, s, NCH_3).[4]

N-Phenylmethoxycarbonylsarcosinamide. N-phenylmethoxycarbonyl-sarcosine (40.2 g, 0.18*m*) was dissolved in dry tetrahydrofuran (200 ml) and cooled to -15 °C. N-Ethylmorpholine (22.8 ml, 0.18*m*) was added followed by 2-methylpropyl chloroformate (23.6 ml, 0.18*m*) and stirring continued for 5 min at -15 °C. Aqueous ammonia solution (0.88 S.G., 225 ml, 0.36*m*) was then added cautiously. The mixture was stirred for 20 min at -15 °C and then allowed to warm to ambient temperature.

Solvent was evaporated under reduced pressure at an external temperature of less than 30 °C. The oily residue was partitioned between ethyl acetate (2 × 300 ml) and water (150 ml) and the combined organic extracts washed till neutral with 10% (w/v) aqueous citric acid, then with 10% (w/v) aqueous sodium hydrogen carbonate, finally with two portions of saturated aqueous sodium chloride and then dried over anhydrous $MgSO_4$. After filtration, the solvent was removed *in vacuo* and the residual oily solid was crystallized from ethyl acetate:hexane. The resulting colorless, crystalline solid was recrystallized from ethyl acetate-hexane to give the product; yield 32.4g, 81%; *m.p.* 88-9 °C, R_f 0.37 (methanol:chloroform, 1:19). Found: C, 59.60; H, 6.15; N, 12.85. $C_{11}H_{14}N_2O_3$ requires C, 59.45; H, 6.35; N, 12.60%. δ ($CDCl_3$) 7.38(5H, *s*, C_6H_5), 6.62-6.25 (2H, *m*, NH_2), 5.18 (2H, *s*, ArCH_2), 3.96 (2H, *s*, CαH_2), 3.03 (3H, *s*, NCH_3).

N-Acetylsarcosinamide. N-Phenylmethoxycarbonylsarcosinamide (22.2 g, 0.1*m*) was dissolved in aqueous acetic acid (80% v/v, 250 ml)

[4]The chemical shifts (δ) are given in p.p.m. from internal TMS. The parenthetical terms give respectively, the integrated signal intensity, a description of the region of the spectrum (*s* denotes a sharp singlet, *bs* a broadened singlet and *m* a multiplet) and the italicized H indicates the assignment of the signal.

and shaken overnight with hydrogen (1 atm) in the presence of 5% palladium-charcoal catalyst[8] (0.5g). After hydrogen uptake was adjudged to be complete, the solution was filtered, the solvent removed, cooled to -15 °C, and added with stirring to a precooled mixture (-15 °C) of acetic anhydride (10.4 ml, 0.11*m*) and pyridine (100 ml). After further stirring (15 min) at -15 °C solvent was evaporated under reduced pressure at minimal temperature. The resulting oil was lyophillised with toluene and then with ethyl acetate until crystallization was induced. The colorless crystalline *product* was recrystallized from ethanol-ether to constant m.p.; yield 12.1g, 93%: *m.p.* 141-2 °C, R_f 0.30 (chloroform:methanol:acetic acid: water, 120:18:1:1.5). Found: C, 46.40; H, 7.65; N, 21.70. $C_5H_{10}N_2O_2$ requires C, 46.15; H, 7.75; N, 21.50%. δ (d_6-DMSO) 7.51 (0.33H, *s*, N*H*), 7.36 (0.67H, *bs*, N*H*), 7.14 (0.33H, *s*, N*H*), 7.02 (0.67H, *bs*, N*H*), 3.91 (0.8H, *s*, CαH_2), 3.87 (1.22H, *s*, CαH_2), 2.98 (2.0H, *s*, NCH_3), 2.79 (1.0H, *s*, NCH_3).

N-Acetyl-L-valinamide has been previously synthesized.[1] The syntheses currently used for N-acetylglycinamide, N-acetyl-L-alaninamide and N-acetyl-L-leucinamide will be described elsewhere.[9] Analytically pure, crystalline materials were thoroughly dried *in vacuo* over P_2O_5 prior to use. All solutions were prepared using glass distilled water which was subsequently deionized.

3. RESULTS

The thermodynamic formalism which is used for the treatment of the enthalpy of dilution results has been described previously[1-5,10] and is based on the concept of excess thermodynamic functions.[11-15] The excess enthalpy (H^{ex}) of a solution containing 1 kg of solvent and two solutes, A and B, may be represented as a polynomial expansion,

$$H^{ex} = h_{AA}m_A^2 + h_{BB}m_B^2 + 2h_{AB}m_Am_B + h_{AAA}m_A^3 + h_{BBB}m_B^3 + 3h_{AAB}m_A^2m_B + 3h_{ABB}m_Am_B^2 + \text{higher order terms} \quad (1)$$

In this equation m_i is the molality of solute i and h_{ijk} is the enthalpic interaction coefficient of the subscripted species. Equation (1) may be generalized to

$$H^{ex} = h_2m^2 + h_3m^2 + \ldots \quad (2)$$

where m(the osmolality) $= m_A + m_B$ and

Table I. Experimental Enthalpies of Dilution at 25 °C

m mol-kg^{-1}	$10^3 n$ mol	m' mol-kg^{-1}	$10^3 q$ J	$10^3 \Delta^a$ J
		SAR		
1.0111	1.8503	0.4931	-137.5	1.7
1.0111	1.8104	0.3189	-185.7	-3.7
1.0111	3.5269	0.6361	-191.6	0.5
1.0111	1.0694	0.2079	-122.6	2.1
0.6624	1.8190	0.4394	-58.6	0.3
0.6624	0.8877	0.2100	-58.4	-0.1
0.6624	0.6438	0.1305	-48.4	1.4
0.3244	0.6090	0.1578	-14.0	0.7
0.3244	1.2950	0.2201	-19.7	-0.1
0.3244	0.5763	0.1006	-19.6	-0.8
0.3244	0.3052	0.0610	-11.5	0.2
		SAR + GLY $y_A{}^b = 0.4955$		
1.0021	1.8773	0.3218	18.6	3.9
1.0021	1.8901	0.4889	7.3	-3.9
1.0021	0.9815	0.1998	13.0	3.9
1.0021	2.1560	0.5365	7.4	-4.2
		SAR + ALA $y_A = 0.50$ ł		
0.7996	1.5075	0.3820	-146.6	1.6
0.7996	1.4830	0.2618	-189.1	-1.3
0.7996	2.9907	0.5200	-197.1	-0.3
0.7996	0.8144	0.1624	-121.0	ı.2
		SAR + ALA $y_A = 0.5014$		
0.4994	0.9903	0.2504	-56.6	1.5
0.4994	0.7920	0.1706	-60.7	0.6
0.4994	1.4722	0.3278	-60.9	-1.4
0.4994	0.4859	0.0978	-46.4	-0.5
0.4994	0.8273	0.1662	-66.4	-1.5
		SAR + VAL $y_A = 0.5009$		
0.2785	0.5637	0.1392	-54.8	-0.4
0.2785	0.8247	0.1847	-52.2	1.3
0.2785	0.4067	0.0919	-53.3	-0.8
0.2785	0.2887	0.0581	-43.6	0.4
0.2785	0.4730	0.0996	-59.0	-0.4
		SAR + LEU $y_A = 0.5102$		
0.7963	1.1130	0.3875	-397.7	0.1
0.7963	2.1636	0.5077	-545.1	0.9
0.7963	1.0905	0.2475	-523.9	-0.6
0.7963	0.7502	0.1506	-423.4	0.1
0.7963	1.7431	0.4411	-541.8	-0.5

[a] Δ is the difference between the observed enthalpy of dilution and that calculated from the results of the least-squares fit. [b] y_A is the solute mole fraction of SAR.

Table II. Excess Enthalpy of Interaction Parameters for the Peptide Systems Investigated at 25 °C

Solute A	Species B [a]	h_2 [b]	$10^3\,\sigma$ [c]	y_A [d]
SAR	SAR	145.2 (1.5)	1.6	-
SAR	GLY	-11.5 (7.1)	4.6	0.4955
SAR	ALA	235.4 (1.9)	1.3	0.5008
SAR	VAL	692.0 (14.)	0.9	0.5009
SAR	LEU	874.2 (1.4)	0.6	0.5102

[a] The abbreviations used are given in the text. [b] The number in parentheses represents the 95% confidence limit of the coefficient. Units: J-kg-mol^{-2}. [c] σ is the standard error of the least-squares fit. Units: J-mol^{-1} [d] In the ternary systems, y_A is the solute mole fraction of solute A.

$$h_2 = h_{AA}y_A^2 + h_{BB}y_B^2 + 2h_{AB}y_Ay_B$$

$$h_3 = h_{AAA}y_A^3 + h_{BBB}y_B^3 + 3h_{AAB}y_A^2y_B + 3y_{ABB}y_Ay_B^2 \qquad (3)$$

The form of Eq. (2) is particularly useful if measurements are performed at constant solute ratio *i.e.*, if y_A $(=m_A/m)$ is fixed. The experimental enthalpy change q arising from the dilution of a solution is given by[1,3]

$$q = n(m' - m)(h_2 + h_3(m' + m) + \ldots) \qquad (4)$$

in which n is the total number of moles of solute(s) and m' and m are the osmolalities after and before dilution.

The primary experimental data obtained from the systems studied are presented in Table I. The coefficients of Eq. (4) are given in Table II and were obtained from a least-squares regression routine with the data sets (m', m, n, q). All of the systems considered here required only the pairwise h_2 term in order adequately to represent the data.

4. DISCUSSION

The heterotactic enthalpic pairwise coefficients for the interaction of SAR with GLY, ALA, VAL and LEU were obtained from the results in Table II, using Eq. (3) and the appropriate homotactic coefficients.[1] The results obtained are presented in Table III.

The homotactic interaction coefficient of SAR was obtained

Table III. Pairwise Interaction Enthalpy Coefficients at 25 °C for Aqueous Systems Containing N-Acetylsarcosinamide

Solute A	Species B	h_{AB}[a]
SAR	SAR	145 (2)
SAR	GLY	18 (19)
SAR	ALA	264 (12)
SAR	VAL	684 (51)
SAR	LEU	851 (49)

[a] The parenthetical term represents the 95% confidence limit and for the ternary solutions was obtained from the confidence limit of the h_2 coefficient and from the confidence limits of the homotactic coefficients. These latter were obtained from Ref. 1. Units: J-kg-mol^{-2}.

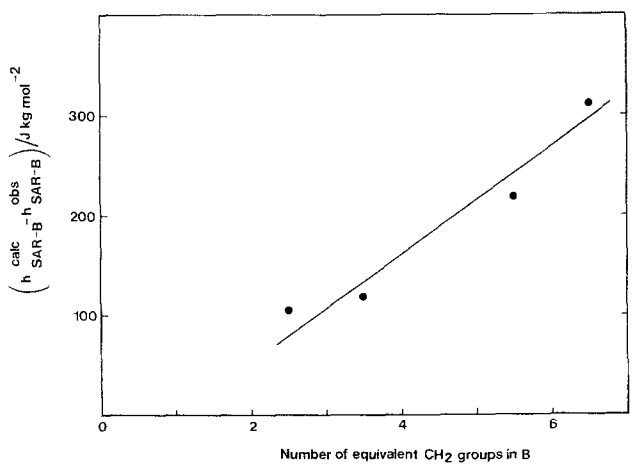


Fig. 1. Comparison of observed and calculated enthalpy coefficients. The calculated coefficients were obtained using the group parameters obtained[4] from systems containing only primary and secondary amide functions.

directly from Eq. (3) and is included in Table III. It is clear from the results (Table III) that the interactions of SAR conform with the previously established[1,4,5] general trends for peptide interactions in that the h_{AB} coefficients became more positive with increasing hydrophobic character of the interacting solutes. This was not unexpected since we presumed that the differences in interactions between secondary and tertiary peptide functions will be relatively subtle.

It has been established[1-5] for the interactions of terminally sub-

stituted amino acids and some dipeptides that the pairwise enthalpic coefficients can be represented rather well using a group additivity approach.[16] This rationalization, which has been used and critically discussed by a number of authors,[1-5,8,9,17-26] postulates that the pairwise thermodynamic coefficient (x_{AB}) representing the interaction of a solute A with a solute B is given, as a first approximation, by

$$x_{AB} = \Sigma\, n_i^A n_j^B X_{ij} \tag{6}$$

Here, n_i^A and n_j^B denote the numbers of functional groups of type i on solute A and type j on solute B respectively and X_{ij} is the intensive term describing the interaction of the groups i and j.

Given of the proven utility of the Savage and Wood additivity of groups (SWAG) scheme with regard to peptide systems,[1-5,8] we have elected to extend this approach in unexpurgated form[5] to systems incorporating the imino peptide function.

In the original formulation[16] and in some later work, methyl, methylene and methyne groups were represented in terms of numbers of equivalent methylene groups while all amide (peptide) groups, primary, secondary and tertiary, were assumed to be equivalent to each other. Using these assumptions the homotactic interaction of SAR is given by

$$h_{SAR\text{-}SAR} = 16H_{CH2\text{-}CH2} + 16H_{CH2\text{-}Pep} + 4H_{Pep\text{-}Pep}$$

and the heterotactic interactions of SAR with N-acetylamides is

$$h_{SAR\text{-}B} = 4(1.5 + n)H_{CH2\text{-}CH2} + (11 + 2n)H_{CH2\text{-}pep} + 4H_{Pep\text{-}Pep}$$

In this last equation n is the number of equivalent methylene groups on the amino acid residue of solute B.

The SWAG parameters $H_{CH2\text{-}CH2}$, $H_{CH2\text{-}Pep}$ and $H_{Pep\text{-}Pep}$ have been derived[4] from a large data set comprised solely of primary and secondary peptide and amide systems. They are currently the best available for such systems and, if the tertiary peptide (iPep) function is equivalent to a proton bearing peptide function (Pep), should predict well the interaction enthalpies for SAR. This was tested using the present data and the previously derived parameters, as shown in Fig. 1. It is

[5]We have recently[4,8] examined alternative formulations of the SWAG approach, but usefully have been unable to improve this scheme for enthalpies of interaction with the currently available data.

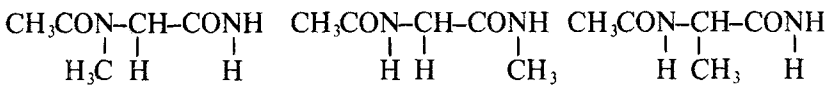
Table IV. Experimental Homotactic Pairwise Interaction Enthalpy Coefficients for Monomeric Peptides of Molecular Formula $C_5H_{10}N_2O_2$

Solute	h_{AA} (J-kg-mol^{-2})
SAR	145
GLYMe	585[a]
ALA	269[b]

[a] From Ref. 4. [b] From Ref. 1.

apparent from this that the discrepancies between predicted and experimental results increases as the side-chain of the amino acid residue increases in number of carbon atoms. There is a similar marked difference between observed (145 J-kg-mol^{-2}) and predicted (512 J-kg-mol^{-2}) values for the enthalpic parameter for the homotactic SAR interaction. Generally we see that the interactions of SAR are enthalpically more favorable than one would expect for a peptide with an equivalent number of CH_2 and Pep groups.

Some insight into the reasons for these differences can be obtained by considering SAR, N-acetyl-N′-methylglycinamide (GLYMe) and ALA.



SAR GLYMe ALA

We shall use the term *isofunctional* to denote a set of molecules whose constitutions lead to the same description when analyzed by the functional group additivity scheme. If we suppose that the interaction parameter of the iPep function is equivalent to that of the Pep function, then SAR becomes isofunctional both with GLYMe and with ALA. The homotactic pairwise enthalpic coefficients of these solutes are given in Table IV.

The more positive value for GLYMe relative to ALA is to be expected from previous observations[1,4,5] and can be justified by the counting procedure used for equivalent methylene groups since ALA contains 3.5 such groups whereas GLYMe contains four. SAR however also has four equivalent methylene groups and the considerably more negative value found for this than found for GLYMe must be a consequence of the presence of the tertiary peptide function

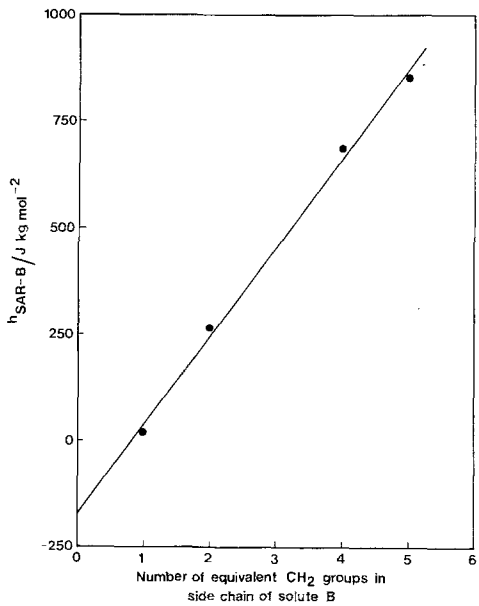


Fig. 2. The heterotactic enthalpic coefficients for the interaction of SAR with the amino acid amides *vs.* the number of equivalent methylene groups in the amino acid side chain. See Eq. (9).

Table V. Enthalpic Pairwise Functional Group Interaction Parameters at 25 °C [a]

i	*j*	H_{ij}
CH_2	CH_2	25.0[b]
Pep	Pep	-291.6[b]
iPep	iPep	-289.0
CH_2	Pep	80.5[b]
CH_2	iPep	28.5
Pep	iPep	-273.2

[a] Units: J-kg-mol^{-2}. [b] Values taken from Ref. 4.

in the sarcosyl derivative.

The data shown in Fig. 1 indicates that the principal source of the discrepancies arises from CH_2-iPep interactions and the qualitative conclusion one can draw is that such interactions will be less positive than those from CH_2-Pep interactions. We have, therefore, quantified this

by extending the original approach so that the tertiary peptide group is considered as a new and distinct functional group in the group additivity scheme. We continue with the earlier assumptions regarding the counting of equivalent methylene groups and that primary and secondary peptide functions behave alike, although it has been shown[4] that this latter is only a moderately good approximation.

The general expression for the enthalpic coefficient of two interacting solute species comprised of CH_2, Pep and iPep groups is thus, from Eq. (6)

$$\begin{aligned} h_{AB} = {} & n^{A}_{CH2} n^{B}_{CH2} H_{CH2\text{-}CH2} + n^{A}_{Pep} n^{B}_{Pep} H_{Pep\text{-}Pep} + n^{A}_{iPep} n^{B}_{iPep} H_{iPep\text{-}iPep} \\ & + (n^{A}_{CH2} n^{B}_{pep} + n^{A}_{Pep} n^{B}_{CH2}) H_{CH2\text{-}Pep} + (n^{A}_{CH2} n^{B}_{iPep} + n^{A}_{iPep} n^{B}_{CH2}) H_{CH2\text{-}Pep} \\ & + (n^{A}_{pep} n^{B}_{iPep} + n^{A}_{iPep} n^{B}_{Pep}) H_{Pep\text{-}iPep} \end{aligned} \tag{7}$$

If limited to the heterotactic interactions of SAR with the compounds studied, Eq. (7) simplifies to

$$\begin{aligned} h_{SAR\text{-}B} = {} & 4(1.5 + n) H_{CH2\text{-}CH2} + 2H_{Pep\text{-}Pep} + (9.5 + n) H_{CH2\text{-}Pep} \\ & + (1.5 + n) H_{CH2\text{-}iPep} + 2H_{Pep\text{-}iPep} \end{aligned} \tag{8}$$

This can be rearranged to

$$\begin{aligned} h_{SAR\text{-}B} = {} & (6H_{CH2\text{-}CH2} + 2H_{Pep\text{-}Pep} + 9.5H_{CH2\text{-}Pep} + 1.5H_{CH2\text{-}iPep} \\ & + 2H_{Pep\text{-}iPep}) + (4H_{CH2\text{-}CH2} + H_{CH2\text{-}Pep} + H_{CH2\text{-}iPep}) n \end{aligned} \tag{9}$$

Figure 2 shows the heterotactic interaction coefficients treated using Eq. (9) and from this we obtained by least squares analysis, values for the intercept and slope of -172(30) and 209(9) J-kg-mol^{-2}, respectively. (The parenthetical terms are the 95% confidence limits.) These, in conjunction with the previously obtained values[4] of $H_{CH2\text{-}CH2}$, $H_{Pep\text{-}Pep}$ and $H_{CH2\text{-}Pep}$, enables values for $H_{CH2\text{-}iPep}$ and $H_{Pep\text{-}iPep}$ to be evaluated. These new functional group interaction parameters are presented in Table V where we have included for comparative purposes values obtained for other group interactions.

Application of Eq. (7) to the homotactic SAR-SAR interaction gives

$$\begin{aligned} H_{SAR\text{-}SAR} = {} & 16\, H_{CH2\text{-}CH2} + H_{Pep\text{-}Pep} + H_{iPep\text{-}iPep} + 8H_{CH2\text{-}Pep} \\ & + 8H_{CH2\text{-}iPep} + 2H_{Pep\text{-}iPep} \end{aligned} \tag{10}$$

Table VI. Comparison of Experimental and Calculated Enthalpy Coefficients[a]

Solute A	Species B	h_{AB}^{expt}	$h_{AB}^{calc\ b}$	$h_{AB}^{calc\ c}$
SAR	SAR	145	512	145
SAR	GLY	18	122	37
SAR	ALA	264	382	246
SAR	VAL	684	902	664
SAR	LEU	851	1162	873

[a] Units: J-kg-mol^{-2}. [b] Counting iPep ≡ Pep. [c] Counting iPep ≠ Pep.

The unknown $H_{iPep\text{-}iPep}$ parameter may thus be estimated from this homotactic interaction and is also included in Table V.

The linearity exhibited in Fig. 2 is independent of the component coefficients of Eq. (9) and is a striking example of the way in which aqueous monomeric peptide systems may exhibit additivity in their interactions. Clearly the interactions of SAR will be very well reproduced by a SWAG scheme and this is illustrated in Table VI

The value of $H_{CH2\text{-}iPep}$ which we have obtained (Table V) is significantly less positive than that for $H_{CH2\text{-}Pep}$.[4] The values of the group parameters for the various peptide-peptide functionalities are sensibly identical but it appears that the interaction of the tertiary peptide group with the methylene group is the major factor which determines the interactive energetics of α-imino acid species *vis a vis* α-amino acid species.

The enthalpic functional group interaction parameters for the iPep group have been obtained from a rather limited set of data. Indeed the $H_{iPep\text{-}iPep}$ coefficient has been deduced from measurements on a single system. It is thus likely that the actual values of these coefficients will vary somewhat as more systems are incorporated in the data base. However, if the SAR interactions are representative of imino peptides generally then the relative differences between iPep and Pep interactions will remain qualitatively correct.

The enhanced enthalpic favorability of gross interactions arising from the presence of a tertiary peptide bond is rather surprising. Tasker and Wood[19] have noted that their experimental value for the homotactic interaction of N,N-dimethylformamide (DMF) was considerably more positive than that predicted by a SWAG treatment using information derived from a coherent set of amide interactions, but

Table VII. Comparison of Experimental and Calculated Enthalpy Coefficients for N-Disubstituted Amides [a]

Solute A	Species B	h_{AB}^{expt}	$h_{AB}^{calc\ b}$	$h_{AB}^{calc\ c}$
DMF	DMF	737 [d,e]	578	217
DMA	DMA	962 [f]	940	474
DMF	DMA	747 [g]	746	333

[a] Units: J-kg-mol^{-2}. [b] Assuming iPep is equivalent to Pep. [c] Assuming iPep and Pep are not equivalent. [d] Tasker and Wood, Ref. 19. [e] Rouw (Ref. 28) obtained an experimental value of 617 J-kg-mol^{-2} for this system. [f] Kresheck (Ref. 27). [g] Rouw (Ref. 28).

which counted amide functions equally regardless of the degree of substitution. These authors[19] noted also that the homotactic interaction of N,N-dimethylacetamide[17] (DMA) also was enthalpically more unfavorable than their predicted value. It was suggested that the N-disubstituted amide function would interact with another N-disubstituted amide function with a change of enthalpy which was less favorable than that of the corresponding interaction for amides with a lower degree of alkyl substitution. Such a diminished interaction was considered[17] to arise both from the impossibility of *direct* amide-amide hydrogen bonding and from the increased steric hindrance of dipole-dipole interactions for the two tertiary amides.

It is apparent that the data for N,N-dimethylamides are not compatible with our observations upon sarcosyl systems. To highlight this difference, experimental values of the pairwise enthalpic interaction coefficients for some N-disubstituted amides are compared in Table VII with values calculated using the new parameter set. The comparison of these emphasizes the differences between 'simple' amide and peptide systems. It is noteworthy that if one assumes the equivalence of the various amide functions, using the SWAG parameters for peptides,[4] one obtains predicted values of h_{AB} for the 'simple' tertiary amides which are in good agreement with experimental values.

Our analysis of the sarcosyl-peptide interactions has shown that the tertiary peptide function is characterized by CH_2-iPep interactions which are more favorable enthalpically than CH_2-Pep interactions. This is transparently not so for the tertiary amides considered above.

A further indication that SWAG schemes should not be used indiscriminately comes from the work of Cesaro and coworkers[29] on enthalpies of dilution of aqueous *cyclo*(-sarcosyl-sarcosyl-) solutions.

They found the homotactic enthalpic parameter for this cyclic peptide to be 577 J-kg-mol^{-2} and this value differs both from that calculated using a SWAG scheme assuming equivalence of all peptide functions (1069 J-kg-mol^{-2}) and the value derived (39 J-kg-mol^{-2}) when Pep and iPep functionalities are distinguished. There is considerable evidence[8,30,31] to suggest that the interactions of cyclic peptides differ markedly and systematically from those of linear peptides and any direct comparison is almost certainly unwarranted.

In view of the several conflicting threads of evidence on compounds containing tertiary amide or peptide functions, we feel that further comprehensive investigations on such systems should be completed before definitive comment on their interactive properties can be made.

ACKNOWLEDGMENT

We acknowledge support from Roche Products Ltd., SERC and ARC.

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