J.C.S. Faraday I, 1980, **76**, 915–922

Aqueous Solutions Containing Amino Acids and Peptides

Part 11.—Enthalpy of Dilution of Single and Binary Solute Solutions of *N*-Acetylglycine Amide, *N*-Acetyl-L-alanine Amide, *N*-Acetyl-L-valine Amide and *N*-Acetyl-L-leucine Amide at 298.15 K

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Received 25*th June*, 1979

The enthalpies of dilution of aqueous solutions containing *N*-acetylglycine amide (G), *N*-acetyl-L-alanine amide (A), *N*-acetyl-L-valine amide (V) and *N*-acetyl-L-leucine amide (L) and equimolal solutions of G+A, G+V, G+L, A+V, A+L and V+L have been measured at 298.15 K. The results obtained have been used to calculate the pairwise enthalpy coefficients for like–like and like–unlike solute interactions. These coefficients have been compared with the predictions of the group additivity approach proposed earlier by Savage and Wood. The group additivity approach works well considering the experimental error and the standard deviation of the original correlations although it seems likely that some refinement of the group interaction parameters is required.

The self-interaction of amino acids and peptides and their interaction with non-electrolytes and electrolytes has occupied our attention for some time.[1-9] Our principal reason for studying these systems is the hope that such investigations will give insight into the factors affecting protein stability in aqueous systems. The earlier studies indicate that the zwitterionic nature of amino acids and peptides plays an important role in their interactions and, extrapolating these conclusions to proteins, the charge distribution on protein surfaces affects how the protein interacts with its environment. It is apparent from structural studies on proteins that there are regions within the polypeptide chain where charges are absent and the contribution to protein stability from these parts of the protein molecule arises from interactions which are essentially of the non-electrolyte–non-electrolyte type. In most proteins there are regions which seem to be well ordered and in, for example, helical or pleated-sheet arrangements. These would seem to indicate that there are relatively strong specific effects arising from peptide–peptide group interactions contributing to the stability of a given protein backbone conformation. Studies on the association of model compounds in water do not, however, indicate any such specific effects.[10-13]

The object of the present set of investigations is the preparation of compounds of the type $CH_3(CONHCHR)_nCONH_2$ (where R denotes an amino acid side chain) and the determination of the excess thermodynamic properties associated with solute–solute interactions in aqueous systems. This initial study is concerned with the enthalpies of interaction of both like–like and like–unlike solute species but our intention is that the work should be extended to other thermodynamic properties and to larger molecules. Our intuitive feeling is that for relatively small molecules, specific (*e.g.*, site-binding) effects will not be observed but as the peptide chain is increased in length such effects will begin to manifest themselves.

EXPERIMENTAL

Heats of dilution were determined using a LKB batch microcalorimetric system. The calorimeter is essentially the same as that described by Wadsö.[14] As an aid to thermal stability, the calorimeter was equipped with a secondary thermostat and also contained in an air-conditioned room. All measurements were made at 298.15±0.05 K.

PREPARATION AND PURIFICATION OF MATERIALS

N-ACETYL-L-ALANINE AMIDE

The ethyl ester hydrochloride of L-alanine was prepared by reaction of L-alanine with thionyl chloride[15] in anhydrous ethanol at 0°C. The product was recrystallised from ethanol+ether and dried *in vacuo* over KOH pellets; yield 85%, m.p. 75-76°C (lit.[16] m.p. 76°C). The ester was acetylated[17] at 0°C using acetic anhydride in pyridine to give *N*-acetyl-L-alanine ethyl ester as an oil, shown to be homogeneous by t.l.c. (R_f 0.4, in 9 : 1 $CHCl_3$: EtOH). This oil was dissolved in anhydrous ethanol, previously saturated with ammonia, and the solution allowed to stand at 20°C for one day. Solvent was removed under reduced pressure and the product repeatedly crystallised from ethanol+ether to constant melting point, 162°C (lit.[18] m.p. 162°C). Found: C, 45.9; H, 7.5; N, 21.5. Calc. for $C_5H_{10}N_2O_2$: C, 46.15; H, 7.75; N, 21.52%. $[\alpha]_D^{22}-39$ (c 1, EtOH) {lit.[18] $[\alpha]_D^{27}-5.64$ (c 0.78, EtOH)}.

N-ACETYLGLYCINE AMIDE

Ammonolysis of a solution of *N*-acetylglycine ethyl ester in dry ethanol gave the amide in quantitative yield. Recrystallisation from ethanol+ether gave white needles, m.p. 138-139°C (lit.[19] 138-139.5°C); Found: C, 41.5; H, 6.8; N, 24.1. Calc. for $C_4H_8N_2O_2$: C, 41.4; H, 6.9; N, 24.1 %.

N-ACETYL-L-LEUCINE AMIDE

Ammonolysis of a solution of *N*-acetyl-L-leucine methyl ester in dry methanol gave impure *N*-acetyl-L-leucine amide. It was recrystallised from chloroform+petrol (b.p. 40-60°C) as a white crystalline product, m.p. 133-134°C, $[\alpha]_D^{20}-25.6$ (c 1, MeOH) {lit.[20] $[\alpha]_D^{20}+26$ (c 1, MeOH) for *N*-acetyl-D-leucine amide}. Found: C, 55.4; H, 9.3; N, 16.1. $C_8H_{16}N_2O_2$ requires C, 55.79; H, 9.36; N 16.26%.

N-ACETYL-L-VALINE AMIDE

Ammonolysis of a solution of *N*-acetyl-L-valine methyl ester in dry methanol gave the corresponding amide. This was recrystallised from methanol+ether, m.p. 234-236°C (lit.[21] m.p. 235°C). $[\alpha]_D^{20}-15.8$ (c 1, MeOH). Found C, 53.64; H, 8.94; N, 17.94. Calc. for $C_7H_{14}N_2O_2$: C, 53.13, H, 8.92; N, 17.71%.

All materials were exhaustively dried before use. The water used was deionised laboratory distilled water.

RESULTS AND DISCUSSION

The excess enthalpy per kilogram of solvent may be expanded as a power series in solute molalities, *i.e.*,

$$H^{ex} = \sum_i \sum_j m_i^m m_j^n h_{im,jn} \qquad (1)$$

where the summation is over all solute species and all positive integral values of m and n. Eqn (1) takes the following forms for solutions containing one solute or two solutes:

Single solute (A) case:

$$H^{ex} = m_A^2(h_{AA}+h_{AAA}m_A+\ldots). \qquad (2)$$

Binary solute (A+B) case:

$$H^{ex} = m_A^2(h_{AA}+h_{AAA}m_A+\ldots)+m_B^2(h_{BB}+h_{BBB}m_B+\ldots)+ m_Am_B(2h_{AB}+3h_{AAB}m_A+3h_{ABB}m_B+\ldots). \quad (3)$$

These expressions can be written in the same form if we use the osmolality $\mathbf{m} = m_A+m_B$ and write $m_A = y\mathbf{m}$ and $m_B = (1-y)\mathbf{m}$, y being the solute molality fraction, thus

$$H^{ex} = \mathbf{m}^2(h_2+\mathbf{m}h_3+\ldots) \quad (4)$$

where

$$h_2 = [y^2h_{AA}+(1-y)^2h_{BB}+2y(1-y)h_{AB}] \quad (5)$$

and

$$h_3 = [y^3h_{AAA}+(1-y)^3h_{BBB}+3y^2(1-y)h_{AAB}+3y(1-y)^2h_{ABB}]. \quad (6)$$

If we consider the particular situations in which $y = 1$ and $y = 0.5$ then eqn (5) and (6) become:

$$\begin{aligned} &\text{when } y = 1: \\ &\quad h_2 = h_{AA} \\ &\quad h_3 = h_{AAA}, \end{aligned} \quad (7)$$

$$\begin{aligned} &\text{when } y = 0.5: \\ &\quad h_2 = (h_{AA}+h_{BB}+2h_{AB})/4 \\ &\quad h_3 = (h_{AAA}+h_{BBB}+3h_{AAB}+3h_{ABB})/8. \end{aligned} \quad (8)$$

The experimental heat change (q) associated with diluting a solution of osmolality $\mathbf{m}$, containing a kg of solvent by the addition of b kg of pure solvent is given by

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

in which n is the total number of moles of solute ($n = a\mathbf{m}$) and $\mathbf{m}'$ is the osmolality of the solution after mixing [$\mathbf{m}' = a\mathbf{m}/(a+b)$]. The experimental heats of dilution (see table 1) were fitted by a least-squares procedure to eqn (9) and the coefficients obtained along with their 95% confidence limits are given in table 2.

In all of the experiments on binary solute systems the solute molality fractions were 0.5 (within $\approx$ 0.1 %) and consequently after the like–like pairwise interaction coefficients were evaluated [see eqn (7)], the like–unlike coefficients could be obtained from eqn (8). The pairwise enthalpy of interaction coefficients are presented in table 3.

It is clear from the information given in this table that the pairwise parameters vary rather markedly with regard to both sign and magnitude. This immediately indicates that dominant effects from say peptide–peptide interactions are not present. In view of some earlier investigations [10-13] on the interactions occurring between amides in water and the success of the approach developed for these we felt it worthwhile to see how well such a procedure would represent the present results.

This group additivity approach of Wood and co-workers assumes that when two solute molecules interact, all groups on one molecule interact with all groups on the other molecule and that each interaction between groups is characterised by a group interaction parameter. If we apply the earlier approaches to the present results then the pairwise interaction coefficients are given by:

$$h_{AB} = n^A_{CH_2}n^B_{CH_2}H_{CH_2-CH_2}+(n^A_{CH_2}n^B_{CONH}+n^B_{CH_2}n^A_{CONH})H_{CH_2-CONH}+ n^A_{CONH}n^B_{CONH}H_{CONH-CONH} \quad (10)$$

$$h_{AA} = n^{A,2}_{CH_2}H_{CH_2-CH_2}+2n^A_{CH_2}n^A_{CONH}H_{CH_2-CONH}+n^{A,2}_{CONH}H_{CONH-CONH} \quad (11)$$

for unlike and like interactions, respectively.

TABLE 1.—EXPERIMENTAL ENTHALPIES OF DILUTION AT 298.15 K

m/mol kg^{-1}	n/10^{-3} mol	m′/mol kg^{-1}	q/J	Δ^a/10^{-4} J
		N-acetylglycine amide		
1,350 4	2.460 1	0.647 2	0.210 7	−47
1.350 4	1.251 1	0.249 0	0.198 4	+3
1.350 4	4.854 8	1.066 3	0.146 5	+26
1.350 4	1.236 4	0.248 6	0.199 3	+36
0.690 7	1.316 3	0.333 0	0.079 8	−9
0.690 7	0.657 5	0.131 1	0.068 6	+20
0.690 7	2.598 0	0.537 2	0.062 4	−20
0.343 0	0.672 6	0.166 7	0.021 9	−14
0.343 0	0.342 4	0.066 0	0.017 9	−12
0.343 0	1.185 7	0.260 1	0.016 9	−7
0.343 0	0.332 8	0.081 8	0.017 5	+2
		N-acetyl-L-alanine amide		
1.000 4	1.901 7	0.474 8	−0.299 6	+5
0.494 9	0.945 0	0.236 4	−0.069 4	0
0.262 7	0.535 4	0.129 0	−0.019 8	0
1.000 4	3.363 7	0.751 1	−0.256 4	4
0.494 9	0.511 2	0.096 7	−0.058 0	−8
0.262 7	0.251 1	0.049 2	−0.014 1	6
0.841 7	1.624 0	0.400 0	−0.210 4	13
0.841 7	0.776 6	0.152 8	−0.158 5	−35
0.841 7	3.113 4	0.651 2	−0.180 7	−23
0.841 7	0.457 5	0.096 8	−0.093 8	+45
		N-acetyl-L-valine amide		
0.163 7	0.330 8	0.081 8	−0.034 2	0
0.163 7	0.342 9	0.082 8	−0.036 4	−14
0.163 7	0.180 4	0.035 2	−0.029 9	−7
0.163 7	0.654 7	0.129 5	−0.027 4	+9
0.163 7	0.491 6	1.122 7	−0.025 7	−3
0.163 7	0.176 7	0.042 4	−0.028 2	−12
0.158 9	0.169 7	0.033 1	−0.024 4	+25
0.123 2	0.243 0	0.059 1	−0.018 5	+12
0.123 2	0.426 5	0.093 1	−0.016 4	−2
		N-acetyl-L-leucine amide		
0.958 5	1.691 8	0.438 8	−1.798	−1
0.958 5	0.863 7	0.178 1	−1.349	+31
0.958 5	3.242 6	0.740 0	−1.460	−6
0.958 5	0.537 1	0.128 5	−0.885	+42
0.958 5	0.408 4	0.087 4	−1.622	−30
0.473 1	0.912 1	0.230 7	−0.435	−40
0.211 3	0.417 4	0.104 6	−0.083 3	−15
0.211 3	0.212 7	0.042 1	−0.066 5	−12
0.211 3	0.798 0	0.166 4	−0.067 3	−7
0.473 1	1.688 6	0.373 2	−0.033 8	0
		N-acetyl glycine amide+*N*-acetyl-L-alanine amide		
2.001 0	2.983 2	0.775 8	−0.338 0	+28
2.001 0	1.766 3	0.356 1	−0.283 5	−16
2.001 0	6.572 7	1.531 6	−0.396 2	−15
0.888 6	1.606 2	0.412 7	−0.064 7	−10
0.888 6	2.004 5	0.660 1	−0.038 2	−3
0.516 2	0.942 3	0.240 8	−0.016 8	−1
0.516 2	0.727 3	0.144 1	−0.015 6	0

N-acetyl glycine amide + *N*-acetyl-L-valine amide

0.307 7	0.614 0	0.148 8	−0.044 4	−3
0.307 7	0.322 5	0.063 4	−0.034 1	+15
0.307 7	1.185 6	0.240 6	−0.037 3	−13
0.307 7	0.317 3	0.076 7	−0.032 0	+11
0.307 7	0.313 3	0.077 5	−0.031 7	+9
0.307 7	0.593 4	0.147 5	−0.043 9	−10
0.307 7	0.180 1	0.039 0	−0.027 0	−1
0.307 7	0.228 6	0.047 8	−0.027 7	−8
0.307 7	0.792 9	0.220 4	−0.031 0	+3

N-acetyl glycine amide + *N*-acetyl-L-leucine amide

1.291 4	2.285 8	0.599 6	−1.030 4	−73
1.291 4	1.071 2	0.219 6	−0.731 0	+120
1.291 4	1.098 6	0.225 7	−0.760 2	−28
0.591 8	1.048 3	0.276 6	−0.213 1	+7
0.591 8	0.556 9	0.115 7	−0.162 8	+87
0.591 8	2.140 3	0.462 1	−0.181 6	−20
0.274 4	0.541 5	0.133 7	−0.051 7	−24
0.274 4	1.059 6	0.214 7	−0.045 7	−48
0.274 4	0.253 8	0.053 2	−0.037 8	−15

N-acetyl-L-alanine amide + *N*-acetyl-L-valine amide

0.291 3	0.279 6	0.055 3	−0.045 0	−3
0.291 3	0.555 1	0.139 3	−0.057 8	−6
0.291 3	0.275 9	0.069 5	−0.041 0	+4
0.291 3	0.837 6	0.208 8	−0.045 8	+10
0.291 3	1.166 4	0.229 8	−0.048 9	−3

N-acetyl-L-alinine amide + *N*-acetyl-L-leucine amide

0.967 3	1.735 5	0.442 2	−0.858 5	27
0.967 3	0.880 8	0.177 4	−0.669 8	−120
0.967 3	3.364 0	0.741 5	−0.713 1	46
0.967 3	0.874 3	0.222 1	−0.615 7	0
0.452 0	0.855 9	0.213 0	−0.186 5	68
0.452 0	0.417 1	0.084 5	−0.139 4	54
0.293 2	0.589 1	0.143 6	−0.079 6	31
0.293 2	0.265 9	0.054 3	−0.058 1	19

N-acetyl-L-valine amide + *N*-acetyl-L-leucine amide

0.202 0	0.293 8	0.099 3	−0.061 54	−14
0.202 0	0.214 0	0.050 9	−0.045 99	+21
0.202 0	0.599 4	0.149 0	−0.046 67	+5
0.202 0	0.197 1	0.040 3	−0.047 22	+1
0.202 0	0.800 2	0.160 5	−0.050 93	−16
0.202 0	0.212 5	0.051 7	−0.046 93	+5
0.202 0	0.400 4	0.098 3	−0.063 27	−15
0.202 0	0.788 1	0.159 3	−0.048 59	+15
0.202 0	0.098 4	0.021 9	−0.025 24	+11

[a] Δ is the difference between the experimental heat and that obtained from the squares regression using the coefficients given in table 2.

We have included in table 3 the numbers of CH_2 groups ($n^{A}_{CH_2}$ and $n^{B}_{CH_2}$) and the numbers of peptide groups (n^{A}_{CONH} and n^{B}_{CONH}) on the A and B molecules. These numbers were calculated using the same assumptions as those made previously [10-13] (see footnote to table 3). Also given in this table are values for the pairwise coefficients calculated from the group interaction coefficients obtained from the earlier investigation,[10] *i.e.*, $H_{CH-CH_2} = 40$ J kg mol^{-2}, $H_{CH_2-CONH} = 41$ J kg mol^{-2} and $H_{CONH-CONH} = -252$ J kg mol^{-2}.

The agreement between the present results and those predicted is shown in fig. 1. The broken lines in this figure correspond to the standard deviation of the original correlation of Savage and Wood.[10] Within this all of the present results agree considering the experimental error. We find the agreement very striking bearing in mind the fact that the results on which the original correlation was based were obtained on molecules containing one CONH group. Consequently there is only one term from

TABLE 2.—COEFFICIENTS OF EQN (9)

solute A	solute B	h_2/J kg mol^{-2}	h_3/J kg^2 mol^{-3}	h_4/J kg^3 mol^{-4}
G	G	−220±9	48±5	—
A	A	268±44	22±11	—
V	V	1259±44	—	—
L	L	1714±94	434±173	−180±94
G	A	46±42	20±14	—
G	V	452±10	—	—
G	L	647±6	—	—
A	V	677±11	—	—
A	L	945±9	—	—
V	L	1486±31	—	—

The errors correspond to the 95% confidence limits in the values of the coefficients.

TABLE 3.—PAIRWISE ENTHALPY INTERACTION COEFFICIENTS

solute A	solute B	$n^{A}_{CH_2}$ [a]	n^{A}_{CONH} [b]	$n^{B}_{CH_2}$ [a]	n^{B}_{CONH} [b]	h^{expt}_{AB} [c] /J kg mol^{-2}	h^{calc}_{AB} [d] /J kg mol^{-2}
G	G	2.5	2	2.5	2	−220±9	−348
A	A	3.5	2	3.5	2	268±9	56
V	V	5.5	2	5.5	2	1259±44	1104
L	L	6.5	2	6.5	2	1714±94	1748
G	A	2.5	2	3.5	2	68±96	−166
G	V	2.5	2	5.5	2	385±47	198
G	L	2.5	2	6.5	2	547±64	380
A	V	3.5	2	5.5	2	591±65	500
A	L	3.5	2	6.5	2	899±73	722
V	L	5.5	2	6.5	2	1486±133	1406

[a] The number of CH_2 groups on A or B. It was assumed that $CH_3 = 1.5$ CH_2 and $CH = 0.5$ CH_2. [b] The number of peptide groups on A or B. It was assumed that any hydrogens attached to the nitrogen are part of the peptide group. [c] Calculated from either eqn (7) or eqn (8) depending upon whether A and B are the same or different. [d] Obtained using the parameters given in table 9 of ref. (11).

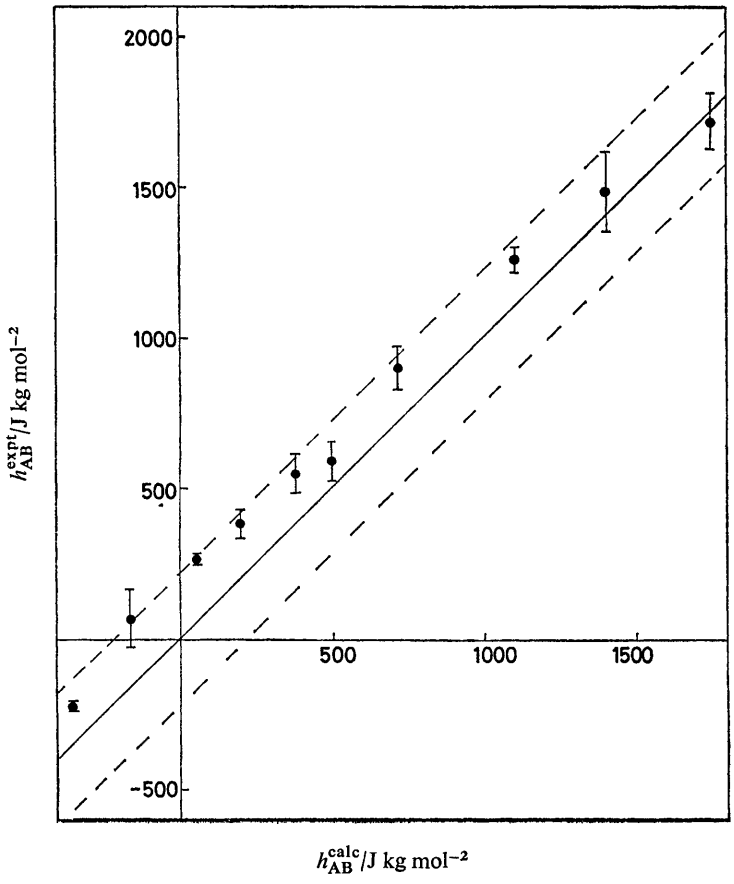

FIG. 1.—Plot of h_{AB}^{expt} against h_{AB}^{calc} for the systems investigated. The calculated values were obtained as described in the text. The broken lines correspond to the standard deviation of the original fit to the data of Savage and Wood.

CONH–CONH interactions in the pairwise enthalpy coefficients of the molecules studied earlier whereas in the present systems there are four such contributions.

Notwithstanding the generally good agreement, fig. 1 shows that there is a trend in the experimental results such that they generally lie above the line obtained from the previous correlation. It is possible that a breakdown of group additivity is occurring because of, for example, nearest neighbour effects, or more likely that the present values for $H_{CONH-CONH}$ and/or H_{CH_2-CONH} are a little in error and need reassessment. We are currently synthesizing peptide derivatives containing three or four CONH groups and it is our intention to use the enthalpy data obtained from these, in conjunction with all of the earlier data, to see if more refined group interaction parameters can be obtained.

The overall success of the group additivity approach for the systems investigated here makes it almost certain that what has been termed " site-binding " is not a contributory factor to the pairwise interactions. In other words, there is no evidence of alignment of the molecules induced by peptide–peptide hydrogen bonding.

We acknowledge support from the S.R.C. and the A.R.C.

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