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Aqueous Solutions Containing Amino Acids and Peptides

Part 17.—Pairwise Enthalpic Coefficients for the Interaction of *N*-Acetyl-L-Phenylalaninamide with some *N*-Acetylamino Acid Amides at 25 °C

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Enthalpies of dilution of *N*-acetyl-L-phenylalaninamide and equimolal solutions of this with *N*-acetylglycinamide, *N*-acetyl-L-alaninamide, *N*-acetyl-L-valinamide and *N*-acetyl-L-leucinamide have been determined using microcalorimetry. The results obtained were used to calculate the pairwise enthalpic coefficients for both like–like (homotactic) and like–unlike (heterotactic) solute interactions. These were then used with a group-additivity approach, which works well for these systems, to obtain information on the interaction of defined groups with each other.

The investigation described here forms part of a continuing study from this laboratory into the interactions occurring between suitably blocked[1–4] amino acids and peptides in aqueous solutions. The motivations for the work have been summarised in these earlier papers. Previous work[1–4] concentrated on the experimental determination and assessment of the interactions occurring in solutions containing the *N*-acetylamides and some *N*-acetylpeptide amides of the amino acids glycine, L-alanine, L-valine and L-leucine. The present study is a fairly straightforward extension of the earlier work and was initiated because we were conscious of the fact that, although the amino acid residues present in the compounds studied earlier were representative of some of the hydrophobic groups present in proteins, the series lacked one of the more important residues in this class,[5] *viz.* the L-phenylalanyl residue. Consequently we have investigated the enthalpies of the homotactic interaction of *N*-acetyl-L-phenylalaninamide (F) and of the heterotactic interactions between F and *N*-acetylglycinamide (G), *N*-acetyl-L-alaninamide (A), *N*-acetyl-L-valinamide (V) and *N*-acetyl-L-leucinamide (L), by the use of enthalpy-of-dilution measurements. It was initially our intention to obtain information on the corresponding free-energetic interactions, but the low solubility of F in water precluded investigations using the apparatus presently available to us. We have, however, initiated the construction of apparatus which will enable such information to be obtained.

EXPERIMENTAL

METHODS

The microcalorimeter used and its ancillary equipment have been described previously.[3,4]

PREPARATION AND PURIFICATION OF MATERIALS

N-ACETYL-L-PHENYLALANINAMIDE

L-Phenylalanine (165 g, 1 mol) was dissolved in 2 mol dm^{-3} sodium hydroxide solution (500 cm^3) in a four-necked flask fitted with two dropping funnels, a thermometer and a stirrer. The whole was cooled to 0 °C. Benzylchloroformate (165.5 cm^3, 1.1 equiv.) and 2 mol dm^{-3}

sodium hydroxide (500 cm^3) were added simultaneously over 45 min and the reaction mixture was then warmed to room temperature.

The viscous liquid was washed with water (500 cm^3) and acidified with 6 mol dm^{-3} hydrochloric acid (200 cm^3). The white precipitate of *N*-benzyloxycarbonyl-L-phenylalanine was filtered, washed with ice water and dried over P_2O_5 (276.7 g, 92.6%) m.p. 88 °C (lit.[6] 88–89 °C); R_F 0.8 (ethylacetate:pyridine:acetic acid:water–240:20:6:11), $\delta(CDCl_3)$ 7.25 (10H, m, C_6H_5), 8.55 (1H, d, $J = 8$ Hz, NH), 5.45 (2H, d, $J = 8$ Hz, $ArCH_2$), 5.28 (1H, m, αCH), 3.36 (2H, m, βCH_2), 3.68 (1H, bs, CO_2H).

N-Benzyloxycarbonyl-L-phenylalanine (59.8 g, 0.2 mol) was dissolved in dry THF (200 cm^3) and cooled to −15 °C. *N*-ethylmorpholine (25.3 cm^3, 0.2 mol) was added followed by isobutylchloroformate (26.4 cm^3, 0.2 mol) and stirred at −15 °C for 5 min to allow formation of a mixed anhydride. Ammonia solution (0.88 S.G., 50 cm^3) was added slowly maintaining a temperature of −15 °C and the mixture stirred for 30 min and then warmed to room temperature.

The solvent was evaporated under reduced pressure and the residue dissolved in ethyl acetate, washed with 5% citric acid (100 cm^3), water (100 cm^3), 5% sodium bicarbonate solution (100 cm^3), saturated brine (100 cm^3), dried over $MgSO_4$ for 20 min, and filtered. Crystallisation was induced by addition of petroleum ether (54.63 g, 93%) m.p. 162 °C (lit.[7] 167 °C), $\delta(CD_3OD)$, 7.25 (10H, m, C_6H_5), 5.0 (2H, d, $J = 5$ Hz, $ArCH_2$), 3.78 (1H, m, αCH), 2.85 (2H, m, βCH_2).

N-Benzyloxycarbonyl-L-phenylalaninamide (14.3 g, 0.05 mol) was dissolved in DMF and hydrogenated overnight in the presence of a Pd/C catalyst (1.5 g). After hydrogen uptake was complete the solution was filtered and cooled to 0 °C.

Dry pyridine (20 cm^3) was added followed by acetic acid anhydride (10.2 g, 0.1 mol). The solution was evaporated to dryness under reduced pressure and the product washed with iced water and crystallised to purity from an ethanol + ether mixture (6.5 g, 70%) m.p. 175–177 °C (lit.[8] 176–177 °C); (found C, 63.80; H, 6.61; N, 13.71; $C_{11}H_{14}O_2H_2$, requires C, 64.06; H, 6.84; N, 13.58), $\delta(D_2O)$ 7.3 (5H, m, C_6H_5) 4.6 (1H, m, αCH) 3.0 (2H, m, βCH_2) 1.94 (3H, s, CH_3CO).

The other compounds used were prepared using minor modifications[9] of the syntheses described earlier.[1]

RESULTS

As in our earlier investigations,[1–4, 10] the enthalpy-of-dilution experiments were treated using a procedure based upon the excess[11–15] thermodynamic function concept.

The excess enthalpy (H^{ex}) of a solution containing 1 kg of solvent may be represented as a polynomial expansion in the solute molality. Using this approach the excess enthalpies of solutions containing only a solute A, only a solute B and containing the two solutes A and B, present in equimolar amounts, are given by

$$H_A^{ex} = h_{AA} m_A^2 + h_{AAA} m_A^3 + \ldots \quad (1)$$

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

$$H_{AB}^{ex} = (h_{AA} + h_{BB} + 2h_{AB}) m^2/4 + (h_{AAA} + h_{BBB} + 3h_{AAB} + 2h_{ABB}) m^3/8 + \ldots . \quad (3)$$

In these equations m_i is the molality of solute i and h_{ijk} is the enthalpic coefficient of the subscripted species. It was mentioned earlier[1] that all three of these equations are of the same general form, *viz.*

$$H^{ex} = h_2 m^2 + h_3 m^3 + \ldots \quad (4)$$

where m is the osmolality, and for the two solutions containing a single solute only, h_2 is equivalent to h_{AA} or h_{BB}. For the solution containing the two solutes

$$h_2 = \tfrac{1}{4}(h_{AA} + h_{BB} + 2h_{AB}). \quad (5)$$

Table 1. Experimental enthalpies of dilution of the systems investigated. In the binary solute systems the solutes were equimolal within 0.1%

$m/10^{-2}$ mol kg^{-1}	$m'/10^{-2}$ mol kg^{-1}	$n/10^{-3}$ mol	$-q/10^{-3}$ J	$\Delta/10^{-4}$ J[a]
		N-acetyl-L-phenylalaninamide (F)		
7.301	4.862	0.2916	7.72	−1.3
7.301	3.637	0.1463	5.93	−1.9
7.301	2.470	0.1488	6.96	2.8
8.383	5.346	0.3193	9.03	2.2
8.383	4.058	0.1610	6.19	7.2
8.383	2.656	0.1599	9.17	−2.4
8.383	6.473	0.3172	5.86	−4.4
8.383	1.934	0.1011	5.93	−0.7
6.494	4.303	0.2640	6.54	−3.7
		N-acetyl-glycinamide + F		
20.027	13.539	0.7799	6.62	−5.7
20.027	6.892	0.4169	6.03	5.2
20.027	9.943	0.3960	4.33	4.4
20.027	16.128	0.7751	4.32	−7.1
20.027	4.115	0.2051	3.78	1.2
		N-acetyl-L-alaninamide + F		
19.926	11.730	0.5518	22.98	−2.6
19.926	6.202	0.3609	25.70	+3.3
19.926	9.816	0.3715	19.73	−5.6
19.926	15.879	0.7759	15.14	+0.8
19.926	14.414	0.5450	14.29	+4.7
		N-acetyl-L-valinamide + F		
20.175	13.573	0.7799	65.40	1.6
20.175	6.381	0.3713	64.97	2.5
20.175	10.213	0.3987	50.01	5.7
20.175	16.384	0.7886	37.42	6.5
20.175	4.037	0.1990	39.95	9.4
—	8.506	0.3544	54.92	−23.0
		N-acetyl-L-leucinamide + F		
20.172	13.481	0.7858	89.19	−13.1
20.172	6.676	0.3979	87.16	26.0
20.172	9.786	0.3858	66.76	2.2
20.172	16.232	0.7816	53.65	−22.1
20.172	3.878	0.1925	52.64	−2.2
20.172	7.521	0.3537	74.94	−1.4

[a] Δ is the difference between the observed and calculated value of the enthalpy change.

Considering the enthalpy change (q) obtained on diluting a solution of osmolality m and containing n moles of solute(s) with pure solvent to give a final solution with osmolality m', it can be shown[1] that

$$q = n(m' - m)[h_2 + h_3(m' + m) + \ldots]. \quad (6)$$

The primary experimental data obtained for the systems studied are presented in table 1, and the coefficients of eqn (6), obtained from using a least-squares fitting routine with these data sets, are given in table 2. For all of the systems studied only the pairwise (h_2) term was needed to represent the data adequately.

Table 2. Coefficients obtained from least-squares analysis of the data in table when fitted to eqn (6)

solutes[a]		
A	B	h_2/J kg mol^{-2}
F	F	1048.6 (52.6)[b]
F	G	119.6 (16.8)
F	A	507.6 (24.2)
F	V	1273.3 (29.7)
F	L	1671.6 (40.1)

[a] The abbreviations used for the amino acid amides are given in the introduction of this paper.
[b] The parenthetical term is the 95% confidence limit of the coefficient.

Table 3. Pairwise enthalpic coefficients of the systems investigated

solute		
A	B	h_{AB}/J kg mol^{-2}
F	F	1049 (53)[a]
F	G	−175 (64)
F	A	356 (82)
F	V	1392 (107)
F	L	1960 (180)

[a] The parenthetical term represents the 95% confidence limit and for binary solute solutions is obtained from the confidence limit of the h_2 coefficient for the binary mixture and from the confidence limits of the component single solute solutions. These latter were obtained from ref. (1).

DISCUSSION

The heterotactic enthalpic pairwise coefficients for the interaction of F with the *N*-acetylamino acid amides were obtained by the use of eqn (5), the information in table 2 and the earlier[1] homotactic coefficient. The results obtained are presented in table 3. The homotactic coefficients for the interaction of F with F is included in this table for completeness. The general trend observed for the h_{AB} coefficients is similar to that found earlier[1] in that as the B species becomes more hydrophobic in character so too does the corresponding enthalpic coefficient become more positive in value.

In our earlier work[1-4] on substituted amino acids and small peptides it was found that in all but one case the pairwise enthalpic coefficients could be represented well by using a group-additivity[16] approach. This approach, which has been used and discussed at some length recently,[1-4, 17-26] postulates that as a first approximation the pairwise thermodynamic coefficient (x_{AB}) representing the interaction of solute A with solute B is given by an expression quadratic in the groups on A and B. The algebraic formulation is

$$x_{AB} = \sum n_i^A n_j^B X_{ij} \qquad (7)$$

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

Given the relative success of the earlier work, we have used the group-additivity ideas with the present systems. It is worth mentioning at this point that there are situations where the group-additivity approach clearly does not work well, and indeed we have shown in one of our earlier papers[3] that for the substituted peptide, *N*-acetyl-L-alanyl-L-alanyl-L-alaninamide, the difference between the experimental and expected enthalpic coefficients is very large indeed. We are also currently investigating[27] systems containing di- and tri-peptides, where the preliminary results clearly indicate marked departures from group additivity. However, our experience so far indicates that for molecules which are not too large or have obvious stereochemical constraints, the group-additivity approach works tolerably well, notwithstanding its simplicity or the fact that its theoretical basis has been adversely criticised.[28,29] (Our general opinion is that given the lack of knowledge of the features which contribute to solute–solute intermolecular events any approach which has predictive and rationalising utility is helpful and should be investigated.) As earlier, the methyl, methylene and methine groups are represented as numbers of equivalent CH_2 groups and both primary (—$CONH_2$) and secondary (—CONH—) amide groups are counted equally. The aromatic residue C_6H_5 is taken as a distinct and new group. The general expression for the enthalpic coefficient of two solute species comprised of equivalent CH_2, Pep and Phe groups is then, from eqn (7),

$$\begin{aligned} h_{AB} = {} & n^{A}_{CH_2} n^{B}_{CH_2} H_{CH_2\text{-}CH_2} + n^{A}_{Pep} n^{B}_{Pep} H_{Pep\text{-}Pep} + n^{A}_{Phe} n^{B}_{Phe} H_{Phe\text{-}Phe} \\ & + (n^{A}_{CH_2} n^{B}_{Pep} + n^{B}_{CH_2} n^{A}_{Pep}) H_{CH_2\text{-}Pep} + (n^{A}_{CH_2} n^{B}_{Phe} + n^{B}_{CH_2} n^{A}_{Phe}) H_{CH_2\text{-}Phe} \\ & + (n^{A}_{Pep} n^{B}_{Phe} + n^{B}_{Pep} n^{A}_{Phe}) H_{Phe\text{-}Pep}. \end{aligned} \quad (8)$$

When we consider the application of this equation to the present heterotactic interactions, it simplifies to give

$$\begin{aligned} h_{FB} = {} & 3(1.5+n) H_{CH_2\text{-}CH_2} + 4H_{Pep\text{-}Pep} + 2[(1.5+n)+3] H_{CH_2\text{-}Pep} \\ & + (1.5+n) H_{CH_2\text{-}Phe} + 2H_{Phe\text{-}Pep} \end{aligned} \quad (9)$$

where n is the number of equivalent CH_2 groups in the amino acid residue (1 for G, 2 for A, 4 for V, 5 for L). Application of eqn (8) to the homotactic F–F interaction gives

$$H_{FF} = 9H_{CH_2\text{-}CH_2} + 4H_{Pep\text{-}Pep} + H_{Phe\text{-}Phe} + 12H_{CH_2\text{-}Pep} + 6H_{CH_2\text{-}Pep} + 4H_{Phe\text{-}Pep}. \quad (10)$$

Eqn (9) can be rearranged to give

$$\begin{aligned} h_{FB} = {} & (4.5H_{CH_2\text{-}CH_2} + 4H_{Pep\text{-}Pep} + 9H_{CH_2\text{-}Pep} + 1.5H_{CH_2\text{-}Phe} + 2H_{Phe\text{-}Pep}) \\ & + (3H_{CH_2\text{-}CH_2} + 2H_{CH_2\text{-}Pep} + H_{CH_2\text{-}Phe}) n \end{aligned} \quad (11)$$

which is linear in the number of equivalent CH_2 groups in the amino acid side chain of B. Fig. 1 shows the experimental data treated in this way and from this we obtained, by least-squares analysis, values for the intercept and slope of -708 J kg mol^{-2} and 1945 J kg mol^{-2}, respectively. Using these along with the earlier values[4] for $H_{CH_2\text{-}CH_2}$, $H_{Pep\text{-}Pep}$ and $H_{CH_2\text{-}Pep}$ enables values for $H_{CH_2\text{-}Phe}$ and $H_{Phe\text{-}Pep}$ to be obtained. These are included in table 4. Substitution of all of these values into eqn (10) then enables an estimate of $H_{Phe\text{-}Phe}$ to be obtained. This is also included in table 4. Note that, although the precise values of the coefficients representing the group interactions might well change as more systems are investigated, the linearity shown in fig. 1 is quite remarkable and is independent of the component coefficients of eqn (11). In some

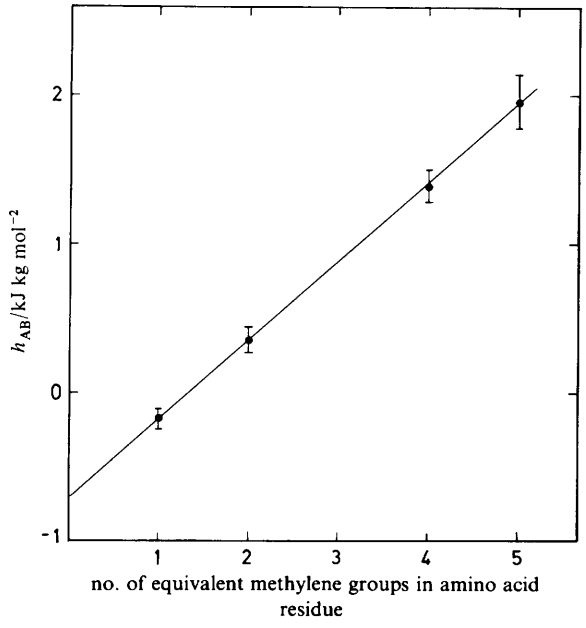

Fig. 1. Plot of the heterotactic pairwise enthalpic coefficient for the interaction of *N*-acetyl-L-phenylalaninamide with *N*-acetylamino acid amides against the number of equivalent methylene groups in the amino acid residue of the latter. The error bars correspond to the 95% confidence limits.

Table 4. Group enthalpic pairwise interaction coefficients

i	j	H_{ij}/J kg mol^{-2}
CH_2	CH_2	25.0[a]
Pep	Pep	−291.6[a]
Phe	Phe	898.2
CH_2	Pep	80.5[a]
CH_2	Phe	294.5
Pep	Phe	−410.2

[a] Taken from ref. (4).

ways it is the strongest evidence yet presented for the applicability of the group-additivity approach to small-solute interactive energetics.

There are some features of the information given in table 4 which are worthy of comment. The sign of the $H_{\text{Phe-Phe}}$ interaction coefficient is positive, as is that for $H_{CH_2\text{-}CH_2}$ and it would appear that this is simply a manifestation of hydrophobic group-hydrophobic group interactions. However, each CH on the aromatic residue is much more efficacious in this regard than a CH group in an aliphatic environment. Roughly speaking the aromatic ring is equivalent to *ca.* six aliphatic CH_2 groups. An estimate of the enthalpic coefficient for the interaction of two benzene molecules in water can be obtained from the work of Tucker *et al.*[30,31] Using their results for the dimerisation of benzene leads to a value for the pairwise enthalpic coefficient of $(8.6 \pm 2.2) \times 10^3$ J kg mol^{-2}, which is *ca.* nine times the value we obtain for two

aromatic rings interacting with each other in peptides containing the phenylalanine residue. The discrepancy illustrates a feature which we felt intuitively[1,3] would be so, namely that group interaction coefficients would be of greatest utility for systems containing molecules of similar basic structure. The idea that groups on side-chains of amino acids need not interact to their maximum possible extent was suggested some years ago by Némethy and Scheraga,[32] and although the general approach adopted by them was quite different to that used by us, the basic concept does seem to be valid. Clearly two benzene molecules could approach each other in such a way as to maximise their possible hydrophobic interaction, whereas the aromatic ring on a phenylalanyl residue has considerably more restrictions in this regard because of the proximity of other parts of the molecule on which it is attached. We shall discuss other manifestations of such steric interactions in later papers in this series[27] when we consider the sequence and stereochemical features observed for some larger peptides.

The value obtained for the interaction of a methylene group with a Phe group is also positive and relatively large, being approximately twelve times that for a CH_2–CH_2 interaction, and it would seem that, probably because of its small size, some optimisation of hydrophobic interactions can result between the CH_2 group and the Phe group.

The most striking result obtained is that for the Pep–Phe interaction, which is large and negative and of opposite sign to that for the Pep–CH_2 interaction. It is premature and possibly dangerous to draw too many conclusions from this enthalpic result on such a small data set but this does seem to indicate a relatively favourable interaction between an aromatic group on one molecule and a peptide group on another molecule. There is some evidence, both from X-ray structural investigations[33,34] and n.m.r. studies,[35–37] that intramolecular associations between aromatic residues and peptide groups are energetically favourable and it does seem reasonable from these to infer that similar intermolecular features would be possible. It would be interesting to carry out an analysis, using a protein structural data base, to see if there is any tendency for intermolecular interactions to occur between aromatic residues and peptide groups. In other words to see if such features contribute to protein architecture.

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