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AQUEOUS SOLUTIONS CONTAINING AMINO ACIDS AND PEPTIDES. PART 18. THE ENTHALPY OF SOLUTION OF *N*-ACETYL-L-PHENYLALANINAMIDE IN AQUEOUS SOLUTIONS CONTAINING FORMAMIDE

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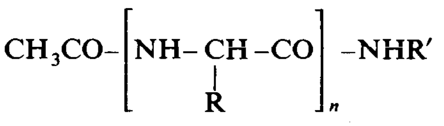
ABSTRACT

The enthalpy of solution at 298.15 K of *N*-acetyl-L-phenylalaninamide (F) in water and in aqueous solutions containing formamide (f) has been determined. The results obtained have been treated to obtain the heterotactic pairwise interaction coefficient for F with f. This disagrees markedly with the value predicted from a Group Additivity approach; reasons for this discrepancy are suggested.

INTRODUCTION

In a series of recent papers [1–5] the energetics of the interactions occurring in aqueous solutions between suitably blocked amino acids and peptides has been investigated. The objective of this investigation is to try to establish some rules which will allow the prediction of the non-covalent bonding interactions which occur when solutes of biological significance are present in water. It has been shown [1–5] that when relatively simple solutes (I) are considered



$$CH_3CO\!-\!\left[\begin{matrix}NH\!-\!\underset{\textstyle R}{\underset{|}{CH}}\!-\!CO\end{matrix}\right]_n\!-\!NHR' \quad \begin{aligned}R &= H,\ -CH_3,\ -CH(CH_3)_2,\\ &\quad -CH_2CH(CH_3)_2,\ -CH_2C_6H_5\\ R' &= -H,\ -CH_3\\ n &= 1\text{–}3\end{aligned} \qquad \text{(I)}$$

a simple Group Additivity [6] scheme rationalises the data quite well.

The primary information which is required are measures of the pairwise coefficients for either homotactic (like–like) or heterotactic (like–unlike) interactions between solute species. These are obtained via the use of the excess thermodynamic function approach and, for example, if the excess enthalpy is considered [1,3] then for a binary solute solution

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

where H^{ex} is the excess enthalpy per kilogram of solvent, m_A and m_B are the molalities of the solutes A and B and h_{ijk} is the enthalpic interaction coefficient for the subscripted species.

The enthalpic information which has been obtained so far [1–5] has come from enthalpy of dilution experiments but in the present investigation a heat of solution procedure has been used. This was adopted partly because the limited solubility of *N*-acetyl-L-phenylalaninamide (F) makes dilution measurements relatively imprecise and tedious, but it was also of interest to see if measurements of this sort could be of general utility in situations where either the solubility is low or material is in short supply.

The object of the present experiments was to investigate the heterotactic interaction between F and formamide (f) in aqueous solutions. We were interested in using the latter since it is the closest one that can approach a molecule which is essentially an isolated peptide group and it was thought to be a useful indicator of whether the group additivity coefficients obtained from the earlier studies [1–5] on substituted amino acids and peptides were transferable to systems containing formamide.

EXPERIMENTAL

The synthesis of F has been described elsewhere [5]. Formamide (B.D.H., AnalaR quality) was further purified by fractional crystallisation.

The enthalpies of solution were obtained using a LKB-8721-1 [7] reaction-solution calorimeter operating at 298.15 K. In each experiment 40–64 mg of F was dissolved in 102.2 cm^3 of solution. The dissolutions were fairly rapid ($<$ 10 min) and the electrical calibrations were made prior to the dissolution experiments. To obtain the corrected temperatures all measured thermistor resistances were converted to temperatures and the initial and final periods fitted by a least-squares procedure to the integrated form of Newton's law of cooling. The temperature changes corresponded to enthalpy changes of 3–4.5 J. Corrections for the heat exchange with the surroundings and the thermal contributions from stirring and Joule heating from the thermistor were made using the Regnault–Pfaundler method. Corrections were also made for the small endothermal contributions arising from evaporations of the calorimetric liquid into the air bubbles released when the ampoules containing F were broken. These latter corrections were estimated to be 0.034 J.

RESULTS AND DISCUSSION

The experimental results obtained are presented in Table 1. The molar enthalpy of solution, ΔH_m, is a function of the molalities of F and f. The functional dependence is given by

$$\Delta H_m = \Delta H_m^\ominus + m_F h_{FF} + m_F^2 h_{FFF} + 2m_f h_{Ff} + 3m_F m_f h_{FFf} + 3m_f^2 h_{Fff} + \ldots \quad (2)$$

where $\Delta H_m^\ominus$ is the standard state molar enthalpy of F in water.

The molalities of F in the final solutions ranged from 2 to 3×10^{-3} mol kg^{-1} and using the earlier value [5] for h_{FF} of 1049 J kg mol^{-2}, and given that the F triplet term is negligible, the maximum contribution from F–F interactions is ~ 3 J mol^{-1} which is well within the experimental precision. Consequently these contributions were neglected as were all other terms in F molality and so eqn. (2) becomes

$$\Delta H_m = \Delta H_m^\ominus + 2m_f h_{Ff} + 3m_f^2 h_{Fff} + \ldots \quad (2a)$$

The data given in Table 1 were fitted to this expression using a least-squares routine and it was found that within the limits of the experimental precision the final term on the right-hand side was not needed. The results obtained were

$$\Delta H_m^\ominus = 13.97 \pm 0.06 \text{ kJ mol}^{-1} \text{ and } h_{Ff} = 47 \pm 26 \text{ J kg mol}^{-2}$$

It is worth commenting that although the value of the latter is small its accuracy as reflected in the 95% confidence limit is comparable with values obtained [1–5] for other systems. As mentioned in the introduction, the earlier data on pairwise interaction coefficients have been treated using a Group Additivity [6,8] scheme. In essence this assumes that every group on solute A interacts with every group on solute B and the net interaction

TABLE 1

Enthalpy of solution of *N*-acetyl-L-phenylalaninamide in water and formamide solutions at 298.15 K

m_f (mol kg^{-1})	ΔH_m (kJ mol^{-1})	s[a] (kJ mol^{-1})	n[b]
0	14.004	0.028	6
0.254	13.994	0.037	4
0.493	13.917	0.038	4
1.003	14.183	0.052	3
1.502	14.026	0.042	4
1.998	14.184	0.047	4

[a] s indicates the calculated standard deviation of the mean.

[b] n indicates the number of experiments.

coefficient is represented as a quadratic function of the groups present, i.e.

$$h_{AB} = \sum_{i,j} n_i^A n_j^B H_{ij} \tag{3}$$

where n_i^A, n_j^B denote the number of groups of type i on solute A and of type j on solute B and H_{ij} is the intensive term representing the i–j interaction. To reduce the number of these intensive terms required to represent relatively limited data sets, the approximation has been made [1–5] that the primary units in the earlier systems are C_6H_5 (Phe), CH_2 and CONH (Pep) and that [6] $CH = 0.5\ CH_2$, $CH_3 = 1.5\ CH_2$, $CONH_2 = CONH$. Using these approximations then the two solutes considered here contain the following equivalent groups

F: 3 CH_2, 2 Pep, 1 Phe

f: 0.5 CH_2, 1 Pep

Substituting these into eqn. (3) gives

$$h_{Ff} = 1.5H_{CH_2-CH_2} + 4H_{CH_2-Pep} + 2H_{Pep-Pep} + 0.5\ H_{CH_2-Phe} + H_{Pep-Phe} \tag{4}$$

The intensive terms in eqn. (4) have been evaluated from the earlier studies ($H_{CH_2-CH_2} = 25.0$, $H_{CH_2-Pep} = 80.5$, $H_{Pep-Pep} = -291.6$, $H_{CH_2-Phe} = 294.5$, $H_{Pep-Phe} = -410.2$ J kg mol^{-2}). Substitution of these into eqn. (4) gives a predicted value for h_{Ff} of -487 J kg mol^{-2} which is quite different from the experimental value. The very large discrepancy clearly indicates the lack of transferability of group coefficients in this case.

It is difficult to be definitive about the reason or reasons for this discrepancy, given our rudimentary knowledge on non-bonding interactions, but we would venture to suggest that it arises from two sources. Firstly if one considers the interaction of what is effectively an isolated Pep group with a polyfunctional molecule then, essentially, all of the Pep group surface area is accessible to the functions in the polyfunctional molecule. However, when the Pep group is itself part of a polyfunctional molecule, necessarily because of intramolecular nearest neighbours effects, its accessible surface area is diminished for interactions with groups on other molecules.

The other reason lies in the nature of the formyl proton on formamide. The indications are [9] that this proton is relatively acidic and consequently can participate via specific hydrogen bonds with both the solvent and suitable hydrogen-bonding acceptor solutes. The consequence of this is that the assumption of the Group Additivity approach regarding the equivalence of all methyne protons would not apply to formyl protons.

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