

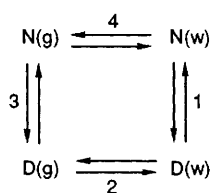
## The Enthalpy of Hydration of Peptide Groups

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A group additivity approach is described for the estimation of the enthalpies of hydration of primary and secondary peptide groups; the values obtained for the two groups are  $-52$  and  $-35$   $\text{kJ mol}^{-1}$  respectively.

The propensity a given protein or enzyme has to fold into its biologically active form is determined by the energetic compromise reached between intramolecular and intermolecular effects. The intramolecular contributions arise from peptide group : peptide group, side-chain : side-chain and peptide group : side-chain interactions, whereas the intermolecular contributions stem from interactions between the peptide groups and the side-chains, with peripheral solvent. The large number and different types of possible non-covalent bonding interactions makes the prediction of protein stability a difficult problem,<sup>1</sup> although the experimental determination of stability has been possible for some time.<sup>2</sup> One approach which has been used<sup>3-7</sup> to compute protein stability addresses the cycle, shown in Scheme 1, where N denotes the native



Scheme 1

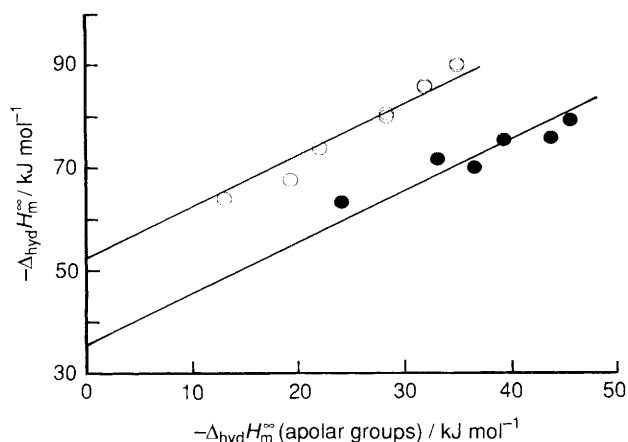
protein, D the denatured protein chain, g the gas phase and w water. Methods are available<sup>1</sup> for the empirical calculation of process 3 and, given that it is the free energy change for process 1 which determines whether or not a protein spontaneously folds in water, information is needed on the hydration of the N and D forms (processes 4 and 2) if the energy change of process 1 is to be evaluated. The present paper considers only one of the many contributions to the overall problem of protein hydration, *viz.* the enthalpies of hydration of primary and secondary peptide groups.

The molar enthalpy changes ( $\Delta_{\text{hyd}}H_m^\infty$ ) for the transfer from the gas phase to water, of some amides of general structures:  $\text{R-CONH-R}'$  and  $\text{R-CONR}'_2$  can be obtained from enthalpies of solution and enthalpies of vaporisation, and appropriate data have been obtained by several groups of workers.<sup>8-15</sup> If it is assumed that the enthalpy of hydration is group additive, then for the primary peptide (secondary amide) and secondary peptide (tertiary amide) groups eqns. (1) and (2) follow. It

$$\Delta_{\text{hyd}}H_m^\infty = \Delta_{\text{hyd}}H_m^\infty(-\text{CONH-}) + \Delta_{\text{hyd}}H_m^\infty(\text{apolar groups}) \quad (1)$$

$$\Delta_{\text{hyd}}H_m^\infty = \Delta_{\text{hyd}}H_m^\infty(-\text{CON}<) + \Delta_{\text{hyd}}H_m^\infty(\text{apolar groups}) \quad (2)$$

should be mentioned that group additivity is not expected to be exact since there must be contributions from intramole-



**Fig. 1** Plot of the molar enthalpies of hydration of amides against the molar enthalpies of hydration of the apolar substituents. The latter were obtained as described in the text. ○ =  $-\text{CONH}-$ ; ● =  $-\text{CON}<$ .

cular electronic effects and particularly from solvational perturbations from peripheral groups.<sup>16</sup> Both of these are neglected in the present approach.

Assuming that the apolar group contributions can be estimated from the enthalpies of hydration of the gaseous parent hydrocarbon and gaseous hydrogen<sup>17,18</sup> (*i.e.* the contribution from, say, an ethyl group is obtained from the difference between the molar enthalpy of hydration of ethane and half the molar enthalpy of hydration of hydrogen).

Fig. 1 shows the experimental results for the enthalpies of hydration of the amides plotted against the enthalpies of hydration of the apolar groups [see eqns. (1) and (2)]. Least-squares analysis gives:  $\Delta_{\text{hyd}}H_m^{\infty}(-\text{CONH}-) = -52.3(\sigma = 2.5)$  kJ mol<sup>-1</sup>;  $\Delta_{\text{hyd}}H_m^{\infty}(-\text{CON}<) = -35.4(\sigma = 2.9)$  kJ mol<sup>-1</sup>. It should be noted that the lines drawn in Fig. 1 have unit slope as required by eqns. (1) and (2).

As expected, both primary and secondary amide groups interact with water exothermically, the enthalpy of hydration of the former being about one and one half times greater than the latter. The obvious deduction to be drawn is that the CONH group forms three hydrogen bonds to water, whereas the CON< group forms two hydrogen bonds to water. Within the precision of the method of estimation used, the bonds to

the  $-\text{CO}$  group and the  $-\text{NH}$  group are of the same strength ( $\sim 17$ – $18$  kJ mol<sup>-1</sup>), although it should be stressed that the values obtained for the enthalpies of hydration are net values and necessarily include contributions from the breaking of hydrogen bonds in the solvent and changes in the van der Waals' contributions.

The success of the procedure used indicates that it should be possible to make reasonably accurate assessments of the enthalpies of hydration of the random-coil forms of proteins and, given a knowledge of the three-dimensional structure and the surface topography of native forms, use the approach to evaluate the enthalpies of hydration of these forms.

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