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Hydration enthalpy of model peptides: N-acetyl amino acid amides

G. Barone [a], G. Della Gatta [b], P. Del Vecchio [a], C. Giancola [a], G. Graziano [a]

[a] *Department of Chemistry, University "Federico II" of Naples, Naples, Italy*
[b] *Department of Inorganic Chemistry, Physical Chemistry and Materials Chemistry, University of Turin, Turin, Italy*

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Abstract

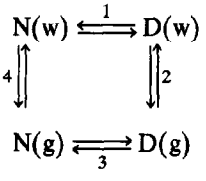
Determination of hydration parameters for the solute–solvent interactions of model peptide molecules can provide quantitative information on the factors affecting the folding and stability of proteins in aqueous solutions. Standard hydration enthalpies are calculated by combination of the standard sublimation and solution enthalpy data, experimentally determined. The results for some N-acetyl amino acid amides, assumed as model for peptides, are reported and the trend of hydration enthalpies with increasing complexity of the model molecules is discussed on the basis of the group additivity method. Further the direct proportionality between hydration enthalpy and non-polar accessible surface area (ASA) of each amino acid residue is emphasized. Finally it is pointed out that there exists a convergence temperature for the enthalpy associated with the hydration process of these model compounds and its value $T_H^* = 93 \pm 7$°C is close to that found for small globular proteins (i.e. $T_H^* = 100 \pm 6$°C). This finding can give some insights to clarify the emergence of convergence behaviour in the unfolding process.

Key words: Hydration; Model peptide compounds; Protein stability; Convergence temperature

1. Introduction

The knowledge of the interactions which stabilize the tertiary structure of globular proteins is the basis for understanding their folding mechanism. Protein stability can be evaluated by different experimental techniques, but its theoretical prediction is a very challenging problem [1]. The complexity of the interactions in the protein molecule, makes it difficult to predict the detailed energetics of the folding–unfolding process. Thus, the study of small model compounds, which mimic the interactions within proteins, can play an important role in providing the thermodynamic information necessary to understand this complex process.

Recently a method has been proposed for computing the effect of the interaction between the protein polypeptide chain and the water molecules on the thermodynamics of protein unfolding [2,3]. The approach is based on the following thermodynamic cycle:



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where N and D represent the native and denatured protein chain in the gas phase (g) and water solution (w). The changes of thermodynamic functions associated with the process 3 can be evaluated by a semiempirical calculation procedure [4], while experimental data for the protein unfolding in aqueous solution can be used to characterize the process 1. Finally, accurate thermodynamic data for the hydration of model compounds are necessary to compute the thermodynamics of the processes 4 and 2. According to this scheme of calculation, a deep investigation of hydration process as is of valuable interest and utility for the general problem of protein folding–unfolding thermodynamics. Detailed studies on the thermodynamic properties of simple hydrophobic compounds are numerous in the literature, concerning the dissolution of either liquid or gaseous apolar compounds into water. However, several authors [5–7] have suggested that a solid model compound system would seem more appropriate in attempting to understand the energetics of protein stability. Packing densities [8] of single protein molecules and their compressibilities in solution [9] suggest that the concept of a 'crystal molecule' [10] is actually operative for the description of the intramolecular interactions and macroscopic properties of these macromolecules. Because of the three-dimensional network of intramolecular interactions, the protein core can be better treated as a solid than as a liquid. To this purpose Murphy and Gill [11–14] have deeply investigated by calorimetry the dissolution process of cyclic solid dipeptides in water, obtaining very significant results for protein thermodynamics.

However, from the point of view of the definition of standard physico-chemical quantities, the hydration process (i.e. gas phase → water solution) gives thermodynamic parameters non-depending on the structure and organization of the starting phase (as occurs for any kind of dissolution or transfer processes). Further the peptide groups in the cyclic dipeptides are in the *cis* configuration, whereas, as it is well known, they assume prevailingly the *trans* configuration in the proteins and naturally occurring polypeptides. The present paper is a contribution along these lines of research. We have determined the standard hydration enthalpies for a set of solid N-acetyl amino acid amides of general formula:

$$CH_3\text{–}CO\text{–}[NH\text{–}CHR\text{–}CO]\text{–}NH_2,$$

where R represents the side chain of an amino acid residue, by using experimentally measured data for solution and sublimation enthalpies. These linear uncharged molecules are very good model compounds to characterize the interactions occurring between amino acid residues and water molecules [15–17]. The experimental results allowed us to obtain an interesting and intriguing connection with some, well established, features of thermal denaturation process of small globular proteins.

2. Experimental

All the substances studied, the N-acetyl-glycine amide (NAGA), N-acetyl-L-alanine amide (NAAA), N-acetyl-L-valine amide (NAVA) and N-acetyl-L-leucine amide, were Bachem products, whose purity was checked by heat of fusion profiles [18]. The standard hydration enthalpy, $\Delta_{hydr}H°$, was calculated from experimentally determined solution and sublimation enthalpies. The enthalpy of solution at infinite dilution in water for the solid samples, $\Delta_{sol}H^{\infty}$, was obtained by means of a CRMT-Setaram rotating calorimeter (Tian-Calvet type). Experimental details and data analysis are reported in the literature [19,20]. The enthalpy of sublimation, $\Delta_{sub}H°$ was obtained by means of a Tian-Calvet type calorimeter equipped with effusion cells, Knudsen type, which allow to measure the enthalpy of vaporization or sublimation for substances whose vapour pressure, at saturation, falls in the range within 10^{-4} and 100 Torr in a wide range of temperature (298–500 K) [21,22]. The measurements were carried out under the very low sublimation equilibrium pressure (in the cell), and in these conditions the gas and solid phases can be considered in their standard states. All sublimation measurements were carried out at 376.15 K, because of the very low vapour pressure of solid samples

studied. No decomposition processes are detectable under the experimental conditions.

A Mettler DSC-20 calorimeter was also used for the determination of heat capacities $C_p°(s)$ of the crystalline solids, in order to transform the enthalpies of sublimation from $T = 376.15$ K to the standard temperature 298.15 K [22].

The temperature scanning assures also that no solid–solid transitions occur in the range 298.15–376.15 K [18]. The molar heat capacities of the gas phase $C_p°(g)$ was calculated by means of an empirical approach, based on the Benson group additivity method that gives satisfactory results in the case of small molecules.

Finally, the standard hydration enthalpy calculated by the difference between solution and sublimation enthalpies, according to the following relation:

$$\Delta_{hydr}H^{\infty}(298.15\ K) = \Delta_{sol}H^{\infty}(298.15\ K) - \Delta_{sub}H^{\circ}(298.15\ K). \quad (1)$$

3. Results and discussion

In Table 1 are given the experimental enthalpies of solution and sublimation and the hydration enthalpy values, calculated according to Eq. (1) for the N-acetyl amino acid amides studied. The hydration enthalpy values clearly appear increasingly negative with increasing the molecular weight. In Fig. 1 are plotted the values of $\Delta_{hydr}H°(298.15\ K)$ are reported against the number of equivalent CH_2 group. The last one is calculated assuming that each CH in the model compound corresponds to 0.5 CH_2 and each CH_3 corresponds to 1.5 CH_2. The values are reported in the last column of Table 2. The obtained linear trend confirms the additivity of functional group contribution. The least-square fitting gives a correlation coefficient of 0.99 and values of -113.8 ± 1.8 and -3.8 ± 0.4 kJ/mol for the intercept and slope, respectively. The slope represents the contribution of each CH_2 group to the total hydration enthalpy and the intercept value is the hydration enthalpy contribution of two CONH groups. In this manner we determined that $\Delta_{hydr}H_{CONH}° = -56.9$ kJ/mol, a value in good agreement with that obtained by Lilley, -52.3 kJ/mol, for the amides [23], and that calculated by Privalov and Makhatadze, -59.6 kJ/mol [24,25]. The negative value of the hydration enthalpy of a CONH group is clearly due to the formation of two or three transient hydrogen bonds in aqueous solution, that are completely absent in the gas phase.

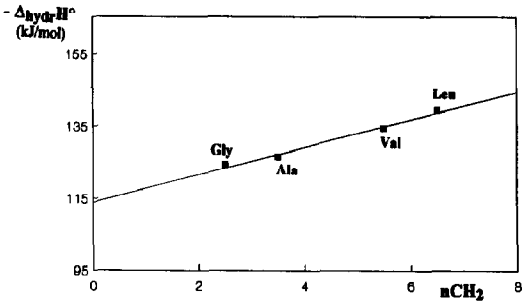

Fig. 1. Plot of the experimental values of $\Delta_{hydr}H°(298.15\ K)$ against the equivalent CH_2 group number for the four N-acetyl amino acid amides.

Table 1
Solution, sublimation and hydration enthalpies for N-acetyl amino acid amides at 298.15 K. Standard deviations are reported in parentheses

Sample	$\Delta_{sol}H^{\infty}$ [a] (kJ/mol)	$\Delta_{sub}H°$ [b] (kJ/mol)	$\Delta_{hydr}H°$ (kJ/mol)
NAGA	15.89 (0.13)	140.2 (2.3)	−124.3 (2.4)
L-NAAA	5.47 (0.17)	131.8 (1.6)	−126.3 (1.7)
L-NAVA	9.29 (0.18)	143.5 (2.2)	−134.2 (2.4)
L-NALA	0.42 (0.02)	139.9 (2.6)	−139.5 (2.6)

[a] Ref. [20].
[b] Ref. [22].

The hydration enthalpy of a CH_2 group is directly determined from the plot slope: $\Delta_{hydr}H_{CH2}° = -3.8 \pm 0.4$ kJ/mol. This negative value can be rationalized in terms of the well-established hydrophobic hydration [26]. Indeed the introduction of an apolar group in water perturbs the solvent structure so that the water molecules optimize their interactions around the apolar surface, strengthening the number and intensity of the hydrogen bonds, and this results in a net exothermic effect. It is worth noting that our calculated value for $\Delta_{hydr}H_{CH2}° = -3.8 \pm 0.4$

Table 2
Equivalent CH_2 group number and hydration enthalpy for N-acetyl amino acid amides at 298.15 K. In the second column are reported the values of non-polar ASA for the corresponding amino acid residue

Sample	*n* CH2	Non-polar ASA (A^2)	$\Delta_{hydr}H°$ (kJ/mol)
NAGA	2.5	45.9 [a]	−124.3 (2.4)
L-NAAA	3.5	80.4	−126.3 (1.7)
L-NAVA	5.5	127.1	−134.2 (2.4)
L-NALA	6.5	156.0	−139.5 (2.6)

[a] The values in the column are from ref. [28].

kJ/mol is in good agreement with that determined by calorimetric measurements on gaseous hydrocarbons by Dec and Gill: −3.4 kJ/mol [27].

To gain further insight on the reliability of our experimental data, we tried to find a relation between the hydration enthalpy values and the surface area accessible to the contact with water molecules. Clearly a relation is expected because the interaction takes place around the surface of solute molecule. Fig. 2 shows a plot of $\Delta_{hydr}H°$(298.15 K) versus the non-polar accessible surface area (ASA) for each amino acid residue as determined by Livingstone et al. [28], using the van der Waals radii calculated by Richards [29]. The values of non-polar ASA for Gly, Ala, Val and Leu are reported in the second column of Table 2. A linear trend is observed in this case also. This result confirms that, at least for the enthalpy contribution, each atomic group interacts with water molecules in proportion to its accessible surface area in the protein structure. This is the basic assumption of a recent method proposed for computing the effect of hydration on folding–unfolding process [2,3]. The linear regression gives a correlation coefficient of 0.983, a slope equal to -0.142 ± 0.017 kJ/mol A^2 and an intercept of -116.5 ± 1.9 kJ/mol. The intercept represents the hydration enthalpy contribution due to the polar groups in the molecules (i.e. two CONH groups) and gives a value for $\Delta_{hydr}H_{CONH}° = -58.3$ kJ/mol, in good agreement with previous determination.

The plot slope represents the hydration enthalpy contribution of each square angstrom of non-polar accessible surface area. It has been shown that the number of apolar hydrogen atoms (i.e. an hydrogen atom bonded to carbon) is proportional to the non-polar ASA [30]. Further, using the Livingstone data, it has been obtained that 14.2 $Å^2$ of non-polar ASA correspond to an apolar hydrogen atom [31]. So it becomes possible to calculate the contribution of the CH_2 group (i.e. two apolar hydrogens) to the total hydration enthalpy: $\Delta_{hydr}H_{CH2}° = -4.0 \pm 0.48$ kJ/mol. Even this value is in good agreement with the previous determination. This estimate is greater than the mean value determined by Makhatadze and Privalov, −3.4 kJ/mol, using a set of normal and branched compounds [25]. It is noticeable that in this set of simple compounds the lower value is shown by the normal alkanes, and that their derivatives (alkanols, amines) show lower values than the corresponding branched compounds.

Our experimental data, analyzed according to two different approaches, give almost the same values for the hydration enthalpy contribution of a polar peptide group CONH and a non-polar CH_2 group. Thus it is suitable to calculate the hydration enthalpies of denatured and native forms of globular proteins, starting form the non-polar and polar contribution additivity and from the values of accessible surface area. It must be remembered that both the hydration enthalpy contributions are negative, confirming that the hydration of amino acid residues is an exothermic

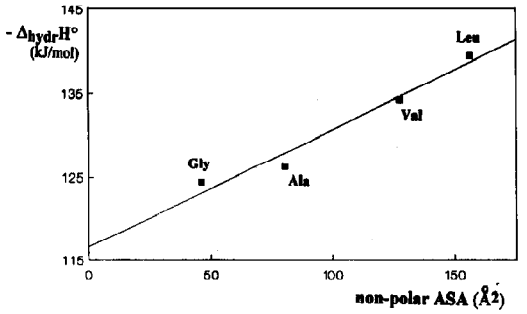

Fig. 2. Plot of the experimental values of $\Delta_{hydr}H°$(298.15 K) of the four N-acetyl amino acid amides against the non-polar accessible surface area of corresponding amino acid residue.

process and, from the enthalpic point of view, tends to destabilize the native folded conformation.

Finally we have tried to demonstrate that even the hydration process of linear solid N-acetyl amino acid amides shows a linear plot of $\Delta_{hydr}H°$ versus $\Delta_{hydr}C_p°$ at 298.15 K. This would indicate that there is a convergence temperature for the enthalpy change associated to this process, labelled as T_H^*. It is well known that convergence temperatures T_H^* and T_S^* for the enthalpy and entropy changes respectively, have been found for a variety of physico-chemical processes [32]: (a) dissolution into water of alkane gases; (b) transfer into water of liquid non-polar hydrocarbons; (c) dissolution into water of cyclic solid dipeptides; (d) thermal denaturation of small globular proteins. In the latter case, when the experimental values of $\Delta_d H°$ and $\Delta_d S°$, normalized per mole of residue, are extrapolated to high temperatures, assuming $\Delta_d C_p°$ as constant, the enthalpy and entropy changes reach constant values [33], common to all proteins in the series at $T_H^* = 100 \pm 6$°C and $T_S^* = 112 \pm 1$°C, respectively. These are the best current estimates of the two convergence temperatures for thermal denaturation of globular proteins [34]. The physical interpretation of the existence of the convergence temperatures for small globular proteins, their different values, although of few degrees, the type of physico-chemical process and model compounds most reliable to describe and rationalize this intriguing phenomenon, are subjects of a very extensive debate between the involved scientists in the last years [35,36].

To accomplish our purpose of constructing a plot of $\Delta_{hydr}H°$ versus $\Delta_{hydr}C_p°$, experimental values of $\Delta_{hydr}C_p°$ (298.15 K) are required for the N-acetyl amino acid amides. These experimental values do not exist in the literature, but we have considered it allowable to calculate them on the assumption of the validity of group contribution approach. Assuming that each N-acetyl amino acid amide molecule is composed of two CONH groups and a variable number of apolar hydrogens (N_{CH}), reported in Table 3, we have used the values of $\Delta C_{p\,CONH}° = -60$ J/K mol and $\Delta C_{p\,CH}° = 28$ J/K mol. These figures have been determined by Murphy and Gill from their calorimetric data of the dissolution process of cyclic solid dipeptides [14]. But the same value of the hydration heat capacity for an apolar hydrogen is observed for the transfer of liquid hydrocarbons into water [37–39], for the dissolution of alkane gases [40], and the transfer of 1-alkanols into water [41]. Further we have shown that, analyzing in terms of group additivity contributions, the transfer of N-alkyl amides from organic liquid phase into water [42], one obtains values for $\Delta C_{p\,CONH}°$ and $\Delta C_{p\,CH}°$ practically identical to those determined by Murphy and Gill. It is reasonable to conclude that these two values are of general validity.

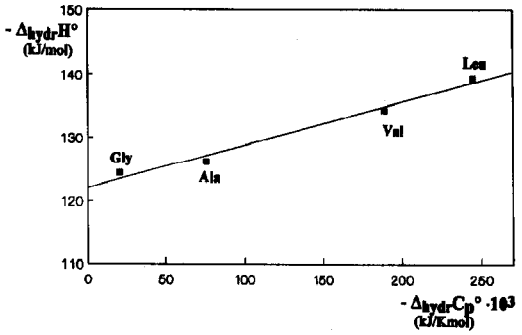

Fig. 3. Plot of the experimental values of $\Delta_{hydr}H°$(298.15 K) versus the values of $\Delta_{hydr}C_p°$(298.15 K), calculated as described in the text, for the N-acetyl amino acid amides. The straight line represents the linear regression result.

Table 3
Number of apolar hydrogens, hydration heat capacity and enthalpy for the N-acetyl amino acid amides

Sample	N_{CH}	$\Delta_{hydr}C_p°$ (kJ/K mol)	$\Delta_{hydr}H°$ (kJ/mol)
NAGA	5	0.020	−124.3 (2.4)
L-NAAA	7	0.076	−126.3 (1.7)
L-NAVA	11	0.188	−134.2 (2.4)
L-NALA	13	0.244	−139.5 (2.6)

The resulting calculated values of $\Delta_{hydr}C_p°$, the experimental values of $\Delta_{hydr}H°$ and the number of apolar hydrogens for the four N-acetyl amino acid amides are reported in Table 3. These data have been used for drawing the desired plot $\Delta_{hydr}H°$ versus $\Delta_{hydr}C_p°$ reported in Fig. 3, and

subjected to a linear regression with respect to the following equation:

$$\Delta_{hydr}H°(298.15\ K) = \Delta_{hydr}H°(T_H^*) + \Delta_{hydr}C_p°(298.15\ K - T_H^*). \quad (2)$$

The results obtained from least-squares analysis are: the correlation coefficient is 0.991, the slope is equal to -68.4 ± 6.6 K and the intercept is -122.1 ± 1.1 kJ/mol. Clearly the data correspond very well to a straight line, thus confirming the existence of a convergence temperature, the value of which can be calculated directly from the obtained slope:

$$T_H^* = 298.15\ K + (68.4 \pm 6.6\ K) = 366.45 \pm 6.6\ K \equiv 93.4 \pm 6.6°C.$$

Thus the convergence temperature of hydration enthalpies for the N-acetyl amino acid amides is very similar to that determined for small globular proteins (best estimate of $T_H^* = 100 \pm 6°C$). It is worth noting that both the models, most frequently used in literature, show convergence temperatures for $\Delta H°$ far away from that of globular proteins. Indeed the liquid hydrocarbon model, first proposed by Baldwin [43] shows $T_H^* = 28°C$, and the cyclic solid dipeptides model of Murphy and Gill shows $T_H^* = 71.5°C$. In addition, utilizing as a model the transfer process of N-alkyl amides from organic liquid phase into water, we determined $T_H^* = 74.3°C$ [42]. It seems that the hydration process of linear solid N-acetyl amino acid amides is a better model, from a physico-chemical point of view, than the previously proposed models, to describe and rationalize the transfer of amino acid residues from the tightly packed core of native globular proteins into the contact with the solvating water molecules.

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Discussion to the paper by Barone et al.

Comments

By P. Privalov

You compare your T_H^* value for hydration of aliphatic groups (93.4 ± 6.6°C) with the T_H^* value for denaturation of globular proteins (100 ± 6.6°C) and assume that they are in good correspondence. I do not think this comparison makes sense, because polar groups in the native protein are interacting with each other and this should effect the T_H^* value, and also non-polar in proteins are not all aliphatic. According to our estimates, the T_H^* of aromatic groups' hydration is about 130°C [1]. Your T_H^* value is in a good agreement with what we found for the hydration of only aliphatic groups in the globular proteins, 86°C. Surprisingly, you do not refer to it in your paper, although it would interesting because these two values were obtained by different extrapolation procedures. The difference between your and our values is caused, as I guess, by two factors:

(a) In your extrapolation you did not take into account the temperature dependence of the hydration heat capacity increment. I do not think this simplification is justified at present time when this dependence is well known [1].

(b) The hydration effect of CH_2 group at 25°C which you obtained, $-(3.8 \pm 0.4)$ kJ/mol, is somewhat higher than what we gave, $-(3.4 \pm 0.4)$ kJ/mol [1]. You explain this difference using the set of normal and branched compounds in our estimates. This is true. The hydration enthalpy of CH_2 group which we gave is an average of values obtained using various types of model compounds (amides, amines, etc.). It is clear that if we are interested in the hydration effects in proteins which represent a mixture of different types of groups, we need just this averaged enthalpy value but not the value for the normal compounds which has purely academic interest.

Also, I do not understand why you compare your T_H^* value for the net hydration effect (i.e. transfer from the gaseous phase to water and that obtained by Murphy and Gill for transfer of diketopiperazines from the crystalline phase to water. It is clear that transfer from the condensed phase includes also the enthalpy of dissociation of non-polar molecules. From the difference in these T_H^* values one can conclude only that interactions of non-polar groups in the liquid hydrocarbons are stronger than in crystalline diketopiperazines, perhaps because of a strong network of hydrogen bonds in this crystal which does not permit close contacts between the non-polar groups.

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By B.K. Lee

(1) What was the standard state used for the reported enthalpy values? The mention of comparison with the data of Dec and Gill indicate that conventional standard was used. However, Ben-Naim [1] shows that a molarity based standard should be used for phase transfer processes. In the case of the transfer from gas phase to water, the relation between Ben-Naim standard, denoted by superscript *, and the conventional standard, denoted by °, is

$$\Delta H^* = \Delta H^\circ + (1 - \alpha T)RT,$$

where α is the thermal expansion coefficient of

the solution [2]. At $T = 298.15$, $(1 - \alpha T)RT = 2.3$ kJ/mol, which is small but not totally negligible.

(2) In the second paragraph of section 3, it is stated that the hydration enthalpy change is negative because water molecules reorganize to strengthen the number and intensity of hydrogen bonds. However, the primary reason for the negative enthalpy change upon hydration at room temperature is likely to be due to the simple van der Waals interaction between the solute and water [2], which is of course absent in the gas phase. Thus, the enthalpy change for the small molecule transfer from gas to water is expected to be always negative. Similarly, the hydration enthalpy of a protein molecule will also always be negative, whether the protein is in the folded or unfolded state. The hydration enthalpy will be larger in magnitude for the unfolded form than for the folded form simply because the former has larger surface area and correspondingly more van der Waals contact with water. The important, non-trivial question is whether the strength (and number) of intramolecular van der Waals interactions between groups of the folded protein is larger or smaller than the increased intermolecular van der Waals interactions between the unfolded protein and water. Thus the statement that hydration enthalpy of amino acids tends to destabilize folded conformation of a protein is correct in the strictly formal sense, but the sentence may mislead many readers.

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By A. Rashin

Theoreticians often go to the extreme trying to reproduce experimental results. What is your estimate of the accuracy of the experimental measurements of hydration thermodynamics, in particular your own? What would be your recommendation to a theoretician trying to reproduce experimental values from thermodynamics of hydration: at what level of agreement between the theory and experiment would you recommend him to stop because errors in measured values do not warrant attempts to further improve the agreement?

By K.P. Murphy

Your paper presents some very nice results on the hydration of N-acetyl-amino acid amides. I would like to take this opportunity to comment on the striking consistency between your results and those on the dissolution of cyclic dipeptides which I have studied with Stan Gill and to which you refer in your paper. The data which you present, as it includes both dissolution and sublimation studies, permits for the first time a direct comparison of some of the models of protein stability.

As you pointed out, there has been some concern about generalizing the cyclic dipeptide results because the peptide bond is in the *cis* conformation. If one plots your dissolution enthalpies versus the number of apolar hydrogens, N_{CH}, one sees that the slope is nearly identical to that obtained from our studies [1–3]. For the amides it is -1.4 ± 0.8 kJ (mol-CH)$^{-1}$ and for the cyclic dipeptides it is -1.3 ± 0.4 kJ (mol-CH)$^{-1}$. This would seem to indicate that in both systems the hydration enthalpy is of larger magnitude than the van der Waals enthalpy of the apolar groups in the crystal. The intercepts should provide information about the hydrogen bonding groups. It is 20 ± 8 kJ mol^{-1} for the amides and 27 ± 4 kJ mol^{-1} for the cyclic dipeptides. Both types of compounds have two peptides per molecule, so that one can estimate 10 ± 4 kJ mol^{-1} and 13 ± 2 kJ mol^{-1} for the hydrogen bond contribution relative to water. This seems to indicate that the *cis* peptides hydrogen bond is, perhaps, modestly stronger than the *trans* peptide hydrogen bond. Do you have crystal structures of these compounds that might allow a structural interpretation of this difference?

It is also interesting to note that the sublimation enthalpies are practically independent of N_{CH} and suggest a hydrogen bond enthalpy, relative to the gas phase, of 67 ± 4 kJ mol^{-1}, consistent with literature values for amides [4], and considerably larger than the ≈ 40 kJ mol^{-1}, reported for the alcohol $-$OH [4].

One final point. In your manuscript you state that the linear solid N-acetyl amino acid amides are a better model for the protein than others which have been used because the value of T_H^* of

93.4°C is closer to that for small globular proteins. However, the T_H^* of 93.4°C is for the gaseous amide to water transfer. If one uses the ΔC_p values from Table 3, estimated from the cyclic dipeptides results, a T_H^* value of 73.4°C is obtained.

[1] K.P. Murphy and S.J. Gill, Thermodynamics 21 (1989) 903.
[2] K.P. Murphy and S.J. Gill, Thermochim. Acta 172 (1990) 11.
[3] K.P. Murphy and S.J. Gill, J. Mol. Biol. 222 (1991) 699.
[4] G.C. Pimentel and A.L. McClellan, The hydrogen bond (Freeman, New York, 1960).

Responses by G. Barone et al. to Comments

To Privalov

We did not take into account the temperature dependence of the hydration heat capacity changes because the values are not yet experimentally determined (some of us are working in this direction), but build up on the basis of group additivity contribution. Further, the specific polar and apolar values utilized have been usually considered constant by other authors such as Gill, Murphy and Freire.

We have not referred to the Privalov and Makhatadze estimate of $T_H^* = 86$°C for the aliphatic groups for a trivial omission.

Different models have been proposed for theoretical and experimental works concerning the folding/unfolding thermodynamic of globular proteins. Basically two classes of conceptual approaches have been privileged. The first considers the parallel hydration process:

(i) isolated native protein ⇒ native protein in aqueous solution;

(ii) isolated denatured protein ⇒ denatured protein in aqueous solution.

The second class starts from the native protein in aqueous solution and tries to model the transfer of residues from the protein "core" into contact with water molecules, using an appropriate physicochemical process involving small model peptide compounds. We did not want to discriminate between these two approaches, but we only tried to make it evident that the convergence temperature shown by the unfolding enthalpy change of small globular proteins is in fair agreement with that for the hydration process of N-acetyl amino acid amides.

To B.K. Lee

In our calculations we chose the conventional standard state. Undoubtedly, using that of Ben-Naim, as suggested by Lee, a higher value of absolute $\Delta_{hydr}H°$ for the peptide group is obtained (i.e. 1.15 kJ/mol), but the CH_2 hydration enthalpy does not change, being given by the plot slope. Thus, this has no consequences on the calculation that we made for comparison with globular proteins.

The difference (−3.4 against −3.8 kJ/mol CH_2) between our estimate of $\Delta_{hydr}H°$ and that of Privalov is due to our limited but homogeneous set of molecules, half of which have branched side chains.

We have interpreted the negative value of hydration enthalpy of a CH_2 group according to the Nemethy and Sheraga view of a higher strength of hydrogen bonds between water molecules surrounding the inserted apolar group. We have preferred to follow this traditional interpretation of hydrophobic effect, nevertheless we have devoted great attention to Lee's work, which tries to shed a new light on this open question.

To A. Rashin

The uncertainties in our experimental data are reported and fall in the actual range of calorimetric uncertainties. Our idea, however, is that at the present state of the art, it is very difficult to discriminate among theoretical models that, although disconnect the hydration process in very different steps, give numerical results close to each other and the experimental values.

To K.P. Murphy

We substantially agree with the consideration of Dr. Murphy about the amides and cyclic dipeptides. The references for the crystal structures of N-acetyl amino acid amides reported by us are [1–6].

Really many interesting features arise from the packing in the crystals. The main is that the first three peptides share six hydrogen bonds with the neighboring molecules, so a value of 44–48 kJ per H-bond can be deduced, while to each NALA

molecule (whose long side chain is well in contact with other apolar groups) pertain totally only two H-bonds. So other interactions in the crystals (the densities differ by many percents) must be considered.

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[3] R. Puliti, C.A. Mattia, G. Barone and C. Giancola, Acta Cryst. C47 (1991) 1658.
[4] G. Barone, C. Giancola, T.H. Lilley, C.A. Mattia and R. Puliti, J. Thermal Anal. 38 (1992) 2771.
[5] R. Puliti, C.A. Mattia and T.H. Lilley, Acta Cryst. C48 (1992) 709.
[6] R. Puliti, C.A. Mattia and T.H. Lilley, Acta Cryst. C49 (1993) 2173.