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The enthalpy-convergence temperature for the dissolution into water of solid α-amino acids

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Abstract

In this paper it is pointed out that there is a convergence temperature for the enthalpy change associated with the dissolution process into water of four solid α-amino acids: glycine, DL-α-alanine, DL-α-aminobutyric acid and DL-α-norvaline. It is important that the value of the convergence temperature for α-amino acids, $T_H^* = 91.2 \pm 6.3$°C is very close to that determined for the denaturation of small globular proteins, $T_H^* = 100 \pm 6$°C. This result is analysed thoroughly in order to obtain information about the energetics of the protein denaturation process. The analysis points out that there is an energy penalty, due to the dehydration and burial of polar groups, that tends to counterbalance the energy gain due to the formation of polar and dispersive interactions in the close-packed interior of globular proteins.

Keywords: Convergence temperatures; Model compounds; Protein stability

1. Introduction

It is well established [1–4] that for some physico-chemical processes concerning the phase-transfer of organic compounds into water, the associated enthalpy and entropy changes converge to common values at two temperatures, labelled T_H^* and T_S^*, respectively, for all the considered compounds of each class. It has been experimentally verified that the entropy convergence temperature is practically equal for all the investigated processes, $T_S^* = 112$°C [4]. However, the enthalpy-convergence temperature values T_H^* are greatly dispersed. Indeed for the transfer of liquid hydrocarbons to

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water T_H^* amounts to 22 ± 6°C [3], for the dissolution into water of gaseous hydrocarbons T_H^* is 100 ± 9°C [5], and for the dissolution of solid diketopiperazines (cyclic dipeptides), T_H^* is 71 ± 12°C [4]. It is also very important that, for the denaturation process of small globular proteins, the enthalpy and entropy changes, normalized to the number of amino acid residues and considering the net denaturation heat capacity change to be constant, converge to $T_H^* = 100 \pm 6$°C and $T_S^* = 112 \pm 1$°C [2,6,7]. This experimental result encouraged researchers to develop more detailed approaches to the analysis and prediction of the thermodynamics of folding–unfolding transitions of globular proteins [8–12]. Furthermore, there are different interpretations of the physical meaning of these convergence temperatures and, as a consequence, contrasting views of the role played by the various interactions in the stabilization or destabilization of the native protein structure [3,6,8–15]. However, no proposed model of those considering the transfer from a condensed phase into water, i.e. a condensed phase is necessary because globular proteins are tightly packed [16,17], shows a T_H^* value close to that of proteins.

In 1990, Murphy et al. [4] pointed out that convergence phenomena are better emphasized by plotting the entropy or enthalpy changes against the heat capacity changes, at constant temperature, usually 298.15 K. These plots avoid errors associated with long temperature extrapolations of the enthalpy and/or entropy values, with the assumption that the heat capacity change is temperature-independent; Lee called them MPG plots [13]. If convergence temperatures do exist, the MPG plots are linear, according to the well-known thermodynamic relationships

$$\Delta_{tr}S^\circ(298.15\,\mathrm{K}) = \Delta_{tr}S^\circ(T_S^*) + \Delta_{tr}C_p^\circ \ln(298.15/T_S^*) \quad (1)$$

$$\Delta_{tr}H^\circ(298.15\,\mathrm{K}) = \Delta_{tr}H^\circ(T_H^*) + \Delta_{tr}C_p^\circ(298.15 - T_H^*) \quad (2)$$

where the subscript tr represents transfer or conformational transition, i.e. denaturation. The MPG entropy plots all have the same slope because T_S^* is practically equal for all the investigated processes; however, the MPG enthalpy plots have very different slopes.

In this paper, we analyse the enthalpy change associated with the dissolution of solid α-amino acids into water. The determined value of T_H^* is close to that of globular proteins. This result is discussed critically so as to gain information and insights into the energetics of native protein structure stability.

2. The behaviour of solid α-amino acids

We have drawn the MPG enthalpy plot for the dissolution into water of solid glycine and the crystalline racemates of the other three α-amino acids, DL-α-alanine, DL-α-aminobutyric acid and DL-α-norvaline, exploiting the results of detailed calorimetric measurements by Spink and Wadsö [18] and Prasad and Ahluwalia [19]. We selected, in addition to glycine, only α-amino acids with non-polar linear side-chains, because the presence of other functional groups would make the analysis ambiguous. DL-α-norleucine is not present in our set because reliable values of its solution enthalpy

cannot be determined due to its very low solubility and low rate of dissolution into water [19]. Table 1 reports for each compound the number of non-polar hydrogen atoms, N_{CH}, (i.e. the hydrogen atoms bonded to a carbon atom, whether aliphatic or aromatic, and assuming, for instance, that a CH_3 group corresponds to three non-polar hydrogens), and the solution enthalpy and heat capacity values at 298.15 K, $\Delta_{sol}H^\circ$ and $\Delta_{sol}C_p^\circ$, respectively. The positive values of $\Delta_{sol}H^\circ$ suggest that the overall interactions are stronger in the crystal than with water molecules. However, by augmenting the length of the non-polar moiety, the $\Delta_{sol}H^\circ$ values decrease markedly, while the $\Delta_{sol}C_p^\circ$ values increase. This is a very important point.

The least-squares regression gave a linear correlation coefficient $r = 0.991$, an intercept equal to $10.6 \pm 0.6\,kJ\,mol^{-1}$, and a slope of 66.2 ± 6.3 K. Thus a linear relationship does exist and the resulting enthalpy convergence temperature T_H^* is $91.2 \pm 6.3°C$. This value is the highest obtained so far for the dissolution into water of crystalline model peptide compounds. However, it is more similar to that observed for gaseous hydrocarbons than for liquid hydrocarbons. But it is very important to stress that the value of T_H^* for the dissolution of α-amino acids is very close to that determined for the denaturation of small globular proteins. Fig. 1 reports the MPG enthalpy plots for solid α-amino acids and for globular proteins (the thermodynamic data for proteins are from Table 1 of Privalov and Gill's review of 1988 [6], excluding parvalbumin): the two lines are nearly parallel.

Furthermore, the value of $\Delta H(T_H^*) = 10.6 \pm 0.6\,kJ\,mol^{-1}$, corresponding to the contribution of polar interactions [8], is lower than that obtained for cyclic dipeptides, $22.0 \pm 2.3\,kJ\,mol^{-1}$, i.e. each cyclic dipeptide molecule has two CONH groups, but twice that of proteins, $5.64 \pm 0.46\,kJ\,mol^{-1}$ residue [7]. Clearly the polar interactions in the crystals of α-amino acids are stronger than in globular proteins, due to the existence of even direct charge–charge interactions. In solid diketopiperazines, an efficient network of hydrogen bonds and dipolar interactions ensures the relative thermodynamic stability of the crystals. The polar interactions also play an important role in the stability of the secondary and tertiary structures of proteins, as determined experimentally and demonstrated by some authors [20,21]. Indeed, Baldwin and co-workers [20] were able to determine the helix–coil transition energetics of a *de novo*

Table 1
Number of non-polar hydrogen atoms, and the enthalpy and heat capacity changes associated with the solution process of α-amino acids into water at 298.15 K

Substance	N_{CH}	$\Delta_{sol}H^\circ$/ ($kJ\,mol^{-1}$)	$\Delta_{sol}C_p^\circ$/ ($J\,K^{-1}\,mol^{-1}$)
Glycine	2	14.16 ± 0.01[a]	-52 ± 5[a]
		14.07 ± 0.14[b]	
DL-α-alanine	4	8.65 ± 0.13[b]	19 ± 4[a]
DL-α-aminobutyric acid	6	6.64 ± 0.03[a]	76 ± 3[a]
DL-α-norvaline	8	0.30 ± 0.03[b]	150 ± 17[b]

[a] Ref. [18].
[b] Ref. [19].

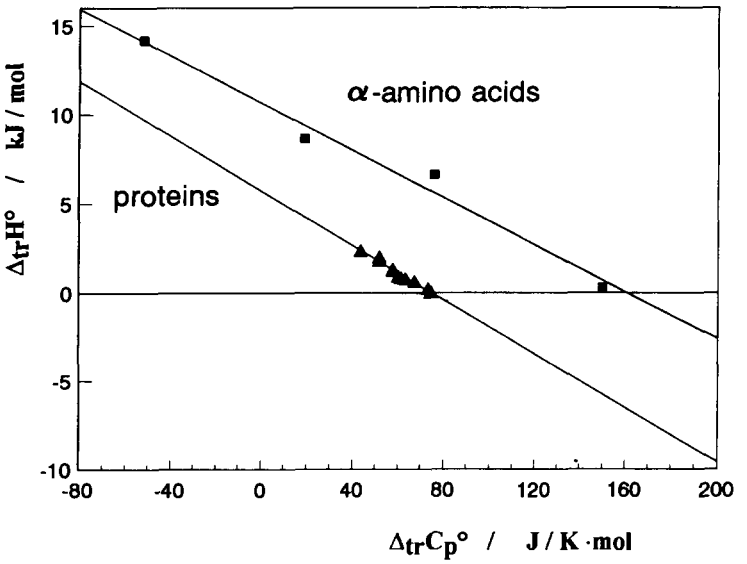

Fig. 1. MPG enthalpy plots for the dissolution into water of solid α-amino acids (data from Table 1), and for the denaturation of 11 globular proteins (data from Table 1 of Ref. [6], excluding parvalbumin).

designed α-helical C-50 alanine-rich polypeptide by direct DSC measurements. Pace and co-workers [21] showed that the deletion of intrachain hydrogen bonds by means of well-designed point mutations strongly destabilizes the native structure of RNAase T1.

According to the group additivity scheme developed by Murphy and Gill [22], the ratio $\Delta_{tr}H^{\circ}_{CH}/\Delta_{tr}C^{\circ}_{pCH}$ corresponds to the slope of the MPG enthalpy plot. The solution enthalpy and heat capacity changes of a CH group, $\Delta_{tr}H^{\circ}_{CH}$ and $\Delta_{tr}C^{\circ}_{pCH}$, respectively, can be determined by least-squares regressions of $\Delta_{sol}H^{\circ}$ and $\Delta_{sol}C^{\circ}_{p}$ against N_{CH}, according to the relationships

$$\Delta_{sol}H^{\circ}(298.15\,K) = \Delta_{tr}H^{\circ}_{pol} + N_{CH}\Delta_{tr}H^{\circ}_{CH} \quad (3)$$

$$\Delta_{sol}C^{\circ}_{p}(298.15\,K) = \Delta_{tr}C^{\circ}_{ppol} + N_{CH}\Delta_{tr}C^{\circ}_{pCH} \quad (4)$$

By performing linear regressions on the values of Table 1, it has been determined that

$$\Delta_{tr}H^{\circ}_{pol} = 18.3 \pm 1.6\,kJ\,mol^{-1};\quad \Delta_{tr}H^{\circ}_{CH} = -2.2 \pm 0.3\,kJ\,mol^{-1};\quad r = 0.983$$

$$\Delta_{tr}C^{\circ}_{pol} = -117.5 \pm 6.2\,J\,K^{-1}\,mol^{-1};\quad \Delta_{tr}C^{\circ}_{pCH} = 33.2 \pm 1.2\,J\,K^{-1}\,mol^{-1};$$

$$r = 0.999$$

The values of the linear correlation coefficients seem to confirm the claimed group additivity. The slope of the MPG enthalpy plot determined from the ratio $\Delta_{tr}H^{\circ}_{CH}/\Delta_{tr}C^{\circ}_{pCH}$ gives rise to a convergence temperature $T^{*}_{H} = 91.3 \pm 6.5$°C, in perfect agreement with the previous estimate. The value of $\Delta_{tr}C^{\circ}_{pCH}$ is large, positive, and

similar to that determined for hydrophobic hydration processes of very different organic compounds [23]. Charged groups, however, make a negative contribution to the heat capacity change, see glycine.

3. Discussion

In this work the denaturation heat capacity change is assumed to be constant, even though Privalov and co-workers have determined, by means of detailed DSC measurements on a set of proteins, that it is temperature-dependent in the range 0–130°C [24–27]. However, this dependence only slightly affects the position and shape of the protein stability curve, i.e. $\Delta_d G^\circ$ versus temperature [28], along the temperature axis, because of enthalpy–entropy compensation effects. Moreover, Makhatadze and Privalov have shown, by considering an extended set of globular proteins and the temperature-dependence of the heat capacity change, that the existence of convergence temperatures for protein denaturation is not as obvious as it appeared previously [29]. In any case the spread of values is not so great as to invalidate the statement that a convergence phenomenon occurs for the denaturation enthalpy and entropy changes of small globular proteins. For this reason, we are interested in a critical comparison of the MPG enthalpy plots of model compounds and protein denaturation, in order to reveal any feature that might elucidate the energetics of protein stability.

The value of $\Delta_{tr}H^\circ_{CH} = -2.2 \pm 0.3\,kJ\,mol^{-1}$ is very large and comparable to the values determined for the hydration of gaseous hydrocarbons, $-2.1 \pm 0.3\,kJ\,mol^{-1}$ [5], gaseous linear alcohols, $-2.0 \pm 0.4\,kJ\,mol^{-1}$ [30], gaseous *N*-alkyl-amides (except the formamide derivatives), $-2.6 \pm 0.2\,kJ\,mol^{-1}$ [31–34], and gaseous *N*-acetyl amino acid amides, $-1.9 \pm 0.2\,kJ\,mol^{-1}$ [35]. For the dissolution of other solid model compounds, the results are: for *N*-acetyl amino acid amides (in the following discussion these compounds are called linear dipeptides for brevity), $\Delta_{tr}H^\circ_{CH} = -1.4 \pm 0.8\,kJ\,mol^{-1}$ [35]; for cyclic dipeptides, $\Delta_{tr}H^\circ_{CH} = -1.3 \pm 0.4\,kJ\,mol^{-1}$ [8] (even though alternative results, obtained recently for five cyclic dipeptides by van de Kleut et al. [36], give $\Delta_{tr}H^\circ_{CH} = -0.8 \pm 0.4\,kJ\,mol^{-1}$). These values are smaller than that found for α-amino acids, but, in any case, are largely negative. However, for the dissolution into water of glycine plus three ω-amino acids (β-alanine, γ-aminobutyric acid and δ-norvaline), the very large value $\Delta_{tr}H^\circ_{CH} = -3.6 \pm 0.3\,kJ\,mol^{-1}$ is found. The values of solution enthalpies at 298.15 K and solution heat capacities at 303.15 K for ω-amino acids are listed in Table 2 and come from the calorimetric measurements of Prasad and Ahluwalia [19]. Fig. 2 shows the plots of $\Delta_{sol}H^\circ$ (298.15 K) vs. N_{CH} for both α- and ω-amino acids. The slope is greater for ω-amino acids, suggesting that the presence and location in the molecules of charged groups has a strong influence on the energetics of the dissolution process into water. Finally and very importantly, it is possible to establish a link with the energetics of conformational transition of globular proteins. Indeed, in the framework of group additivity, from $T^*_H = 100 \pm 6$°C and by considering $\Delta_{tr}H^\circ_{CH} = 30.0\,J\,K^{-1}\,mol^{-1}$, i.e. this value is the average obtained from a very large number of investigated classes of organic compounds [23], the result $\Delta_{tr}H^\circ_{CH} = -2.3 \pm 0.2\,kJ\,mol^{-1}$ is obtained for the denaturation process of small globular pro-

Table 2
Number of non-polar hydrogen atoms, and the values of solution enthalpies at 298.15 K and solution heat capacities at 303.15 K of ω-amino acids

Substance	N_{CH}	$\Delta_{sol}H^{\circ}$/ (kJ mol^{-1})	$\Delta_{sol}C_p^{\circ}$/ (J K^{-1} mol^{-1})
Glycine	2	14.16 ± 0.01[a]	-56 ± 20[a]
		14.07 ± 0.14[b]	
β-Alanine	4	7.99 ± 0.10[b]	-30 ± 7[b]
γ-Aminobutyric acid	6	-0.64 ± 0.06[b]	8 ± 8[b]
δ-Norvaline	8	-6.74 ± 0.09[b]	51 ± 17[b]

[a] Ref. [18].
[b] Ref. [19].

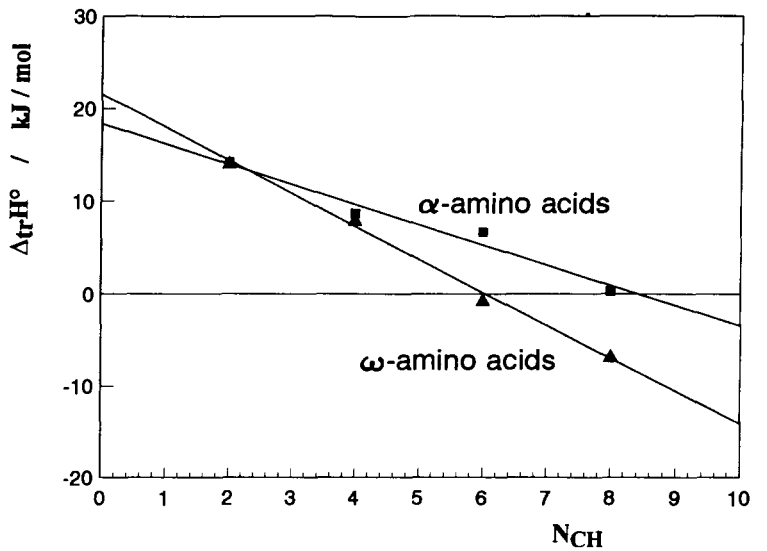

Fig. 2. Enthalpy changes at 298.15 K versus the number of non-polar hydrogen atoms for the dissolution into water of solid α-amino acids (data from Table 1), and ω-amino acids (data from Table 2).

teins. This figure is very close to that obtained for solid α-amino acids and both resemble a 'gas-like' behaviour.

The strong negative values of $\Delta_{tr}H^{\circ}_{CH}$ for the transfer of organic compounds from gaseous phase into water are readily explained by the gain of van der Waals dispersive interactions of the CH group with the surrounding water molecules, interactions that are clearly absent in the gaseous phase, where the molecules are far away. For liquid hydrocarbons, the value of $\Delta_{tr}H^{\circ}_{CH}$is practically zero, i.e. the line of the MPG enthalpy plot is parallel to the x-axis and $T_H^* = 22 \pm 6$°C [3], because the hydrocarbon–hydrocarbon and hydrocarbon–water dispersive interactions have nearly the same

strength, as demonstrated by applications of Scaled Particle Theory [37,38], and detailed theoretical calculations [39]. Furthermore, surface tension measurements of organic-liquid–water systems have shown that the work of adhesion between hydrocarbon and water interfaces is the same as that between two hydrocarbon interfaces [40,41].

The strong negative values of $\Delta_{tr}H^{\circ}_{CH}$ found for the dissolution of solid amino acids, linear and cyclic dipeptides and the denaturation of globular proteins are an unexpected result. A net enthalpic contribution, arising from water reorganization around the solute molecule, is also present, but it should be common to all the dissolution processes, i.e. gaseous hydrocarbons, liquid hydrocarbons, solid amino acids and dipeptides, and globular proteins, and cannot explain the observed differences in the slope of MPG enthalpy plots. Moreover, a positive contribution could be present in the case of gas dissolution in order to create a cavity in the water solvent to accommodate the solute molecule [42,43].

From the enthalpic point of view, it seems that a CH group exerts more favourable interactions with water molecules in solution than with other groups in the solid phase or protein interior. An explanation of this paradoxical conclusion could be that side-chains of α-amino acids are unable to pack optimally. However, this argument fails in the crystal due to the high density of solids with respect to water. The same must occur for globular proteins, which are tightly packed [16, 17]. In addition, the very high density of the protein interior, as revealed by Chothia and co-workers [44], implies that packing interactions play a more fundamental role in protein stability than has hitherto been believed. This result is completely at odds with the strong negative value of $\Delta_{tr}H^{\circ}_{CH}$ obtained from the MPG enthalpy plot of proteins: this quantity should be positive. Honig and co-workers [45, 46] have proposed a different approach to explain the very negative slope of the MPG enthalpy plot. With both arguments and calculations [45–47], these authors suggested that there is an unfavourable enthalpy contribution which arises from the dehydration of polar groups when they are buried in the protein core, and whose relative weight and importance to the overall energy balance increase with increasing $\Delta_d C^{\circ}_p$, assumed to be a measure of protein hydrophobicity. The polar interactions are very strong in the interior of globular proteins or in crystals, but in order to form them, polar groups must be buried and must lose the water molecules in the first hydration shell. Clearly these two effects act in opposing directions: the first tends to stabilize and the other to destabilize the condensed phase or mesophase with respect to water solution. From the positive values of denaturation enthalpies and solution enthalpies of α-amino acids, and linear and cyclic dipeptides, it results that polar and dispersive interactions in globular proteins or in crystals overwhelm those occurring in water. However, with increasing length of non-polar side-chains, the polar interactions in the protein core become weaker because the local packing density decreases and, for instance, the geometric requirements to form strong hydrogen bonds are not satisfied. Therefore, the energetic cost of dehydration becomes more important. In other words, by making the condensed phase more hydrophobic, the positive enthalpy change associated with the burial and dehydration of polar groups tends to counterbalance the negative enthalpy change due to the formation of polar interactions. This results in a decrease in the values of denaturation enthalpy changes,

normalized per residue, on increasing the denaturation heat capacity change: $\Delta_d H^\circ(25°C) = 2.37\,kJ\,mol^{-1}$ residue and $\Delta_d C_p^\circ = 43.5\,kJ\,K^{-1}\,mol^{-1}$ residue for ribonuclease A, and $\Delta_d H^\circ(25°C) = 0.04\,kJ\,mol^{-1}$ residue and $\Delta_d C_p^\circ = 74.5\,kJ\,K^{-1}\,mol^{-1}$ residue for met-myoglobin, respectively the least and most hydrophobic protein considered by Privalov and Gill in their review of 1988 [6]. Similarly, in the crystals of model peptides and amino acids, on increasing the length of the alkyl side-chains, the packing density decreases and finally not all of the potential hydrogen bonds can be established (see, for instance, the structures of *N*-acetyl amino acid amides [48–50]). From this point of view, Eq. (3) is only a rather crude approximation for the analysis of the dissolution from a condensed phase into water, or the denaturation process. The polar contribution to the enthalpy change cannot be assumed as constant along a given series of compounds, but can be split into two terms: $\Delta_{tr}H^\circ_{pol}$, corresponding to the intercept of Eq. (3), represents the energy gain with respect to water solution due to formation of polar interactions when there are no hydrophobic CH groups in the condensed phase, and is always positive; and the second contribution which depends on the hydrophobicity of the medium, i.e. it is a function of $\Delta_{tr}C_p^\circ$, measures the increasing importance of the energy loss due to dehydration of polar groups on increasing $\Delta_{tr}C_p^\circ$, and is always negative. So, the large and negative values obtained for $\Delta_{tr}H^\circ_{CH}$, by applying the group additivity approach to condensed phases, do in fact take into account the strongly favourable interactions of polar groups with water molecules rather than the so-called hydrophobic hydration of non-polar side-chains.

Clearly the destabilizing effect of burying polar groups is only operative in a condensed phase (crystal, pure liquid, protein interior), and is also consistent with the large negative slope of the MPG enthalpy plot for dissolution of solid α-amino acids. Indeed, even though the number of charged polar groups per α-amino acid is constant, the energy cost required to bury these groups from water contact into the crystal would become more important with increasing side-chain length. In the case of ω-amino acids, this behaviour seems emphasized, due to the charge-pair separation on increasing the number of aliphatic carbons. Additivity is likely to hold in water where the solute is dilute and the polar contribution is actually constant. But in the crystal interior, the situation is very different because charged polar groups feel the presence of non-polar moieties closeby. Therefore, the linearity of the MPG enthalpy plot does not necessarily imply that additivity holds and the analysis should be more accurate.

Moreover, the results of Chothia and co-workers [44], on the volume changes on protein folding, demonstrate that the additivity principle must be regarded with caution, because the chain-like nature of proteins imposes restrictions that cannot be modelled by quantities derived from investigations on small molecules. The importance of packing interactions and the non-equality of polar interactions inside the protein and with water have also been advocated by Privalov and Makhatadze [51,52], although these authors used a compact gaseous state as reference and applied a group additivity approach to separate the different forces ensuring the stability of globular proteins. However, Lee, in his analysis of MPG plots [13], stressed that polar interactions play an important role in determining the protein stability and, in consequence, the value of T_H^* close to 100°C, far removed from $T_H^* = 22°C$ found for the transfer of liquid hydrocarbons to water.

In conclusion, it seems that, from the energetic point of view, the denaturation process, considered as the transfer of amino acid residues from the protein interior into contact with water, can be modelled by the dissolution of solid α-amino acids into water. The protein core resembles the crystal of amino acids for the balance between dispersive and polar, i.e. charge–charge, dipolar and hydrogen bond, interactions and the dehydration penalty due to the burial of polar groups. Globular proteins can really be considered as crystal molecules (according to the suggestion of Liquori [53]), or aperiodic crystals (according to the model of Shakhnovich and Finkelstein [54]). However, it is important to bear in mind that they are heteropolymers and their conformations at equilibrium strongly depend on water, in which they are usually dissolved and functionally active.

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