

ENTHALPIES AND ENTROPIES OF FUSION OF SOME SUBSTITUTED DIPEPTIDES

Comparison with crystal data

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Enthalpies and temperatures of fusion or transition for four substituted dipeptides (N-acetylamides of glycyl-*L*-alanine (NAGAA), *L*-alanyl-*L*-alanine (NAA₂A), *L*-prolyl-glycine (NAPGA) and *L*-leucyl-*L*-proline monohydrate (NALPA·H₂O)) were determined by differential scanning calorimetry and the entropies of fusion derived. The results obtained have been compared with those of the corresponding substituted aminoacids and some of their racemic crystalline mixtures. The enthalpies and entropies of fusion of some substituted aminoacids have been redetermined. The results are discussed in comparison with crystal structural data, which has been reported in the literature or determined recently by some of the authors. Rationalization of the fusion parameters was attempted mainly on the basis of the number of intramolecular hydrogen bonds and the packing densities in the crystals.

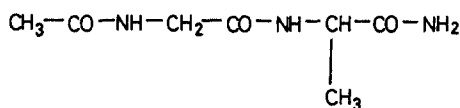
Keywords: enthalpies and entropies of fusion, peptides

Introduction

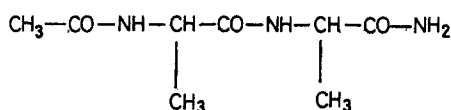
Uncharged derivatives of aminoacids and peptides have been used as model compounds of natural polypeptides and proteins [1-3] in some solution physico-chemical studies. However, thermodynamic and structural properties of the solid phases of these compounds are interesting *per se* and, given the lack of information on this class of compounds, we recently have determined a series of crystal structures [4-9], and sublimation enthalpies and entropies [5, 10, 11]. The structures of a few similar compounds have been reported [12-14].

As part of a program of work on the solid state properties of organic substances which can act as models for the subunit constituents of biological macromolecules, we here report the results of a calorimetric study on the following four uncharged dipeptide derivatives:

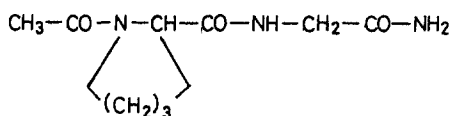
N-acetyl-glycyl-L-alaninamide (NAGAA):



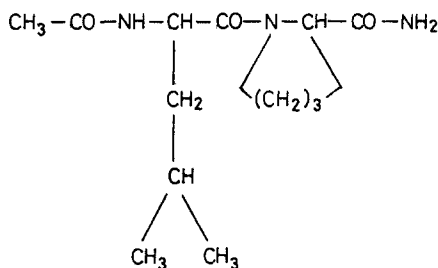
N-acetyl-L-alanyl-L-alaninamide (NAA₂A):



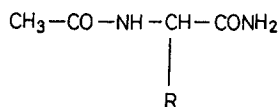
N-acetyl-L-prolyl-glycinamide (NAPGA):



N-acetyl-L-leucyl-L-prolinamide monohydrate (NALPA·H₂O):



Seven N-acetyl amides of primary aminoacids, of general formula:



have also been studied, where

- R = -H (N-acetylglycinamide: NAGA),
- = -CH₃ (N-acetyl-L(or D)-alaninamide: L-NAAA, D-NAAA),
- = -CH(CH₃)₂ (N-acetyl-L-valinamide: L-NAVA),
- = -CH₂CH(CH₃)₂ (N-acetyl-L(or D)-leucinamide: L-NALA, D-NALA),
- = -CH(CH₃)CH₂CH₃ (N-acetyl-L-isoleucinamide: L-NAIA).

Table 1 Thermodynamic results and some crystallographic parameters pertinent to the fusion of N-acetylamides of some aminoacids and peptides

	<i>T</i> K	$\Delta_f H /$ kJ·mol ⁻¹	$\Delta_f S /$ J·mol ⁻¹ K ⁻¹	<i>d</i> / g·cm ⁻³	H-bonds ^a
NAGAA ^b	522.7(0.2)	50.6(0.5)	96.8(1.0)	1.340	4
NAA ₂ A ^b	511.2(0.4)	38.1(0.8)	74.5(1.6)	1.244	4
NAPGA ^{b, c}	434.1(0.1)	32.2(0.4)	73.7(0.5)	1.342	3
NALPA·H ₂ O ^d	349.0(0.2) ^e	61.5(0.4) ^e	—	1.208	3+1.5 ^f
NAGA	410.9(0.1)	27.0(0.1)	65.7(0.3)	1.319	3
	408.2(0.3) ^g	25.6(0.4) ^g			
	412.2(0.1) ^h	26.7(0.1) ^h			
<i>L</i> -NAAA	435.4(0.1)	23.7(0.3)	54.4(0.7)	1.203	3
	431.0(0.4) ^g	21.7(0.2) ^g			
	436.4(0.1) ^h	23.6(0.1) ^h			
<i>L</i> -NALA ⁱ	401.4(0.3)	17.4(0.3)	43.3(0.3)	1.125	2
	404.4(0.1) ^h	16.6(0.1) ^h			
<i>D</i> -NALA	401.4(0.1)	16.2(0.3)	40.3(0.7)	"	"
	401.4(0.1) ^g	20.2(0.3) ^g			
<i>L</i> -NAPA	418.7(0.1)	28.1(0.1)	67.1(0.2)	1.294	2
	417.8(0.2) ^h	28.1(0.2) ^h			
<i>D, L</i> -NAAA	433.6(0.3)	25.3(0.1)	58.3(0.3)	1.168	3
<i>D, L</i> -NALA	401.0(0.2)	2.8(0.1)	7.0(0.2)	1.077	3
<i>L</i> -NAVA	509.0(0.2)	36.9(0.4)	72.5(0.8)	1.135	3
	509.0(0.4) ^j	41.3(0.6) ^j			
	509.7(0.1) ^h	38.1(0.2) ^h			
<i>L</i> -NAIA	529.6(0.2)	41.8(0.1)	78.9(0.2)	—	—

Notes

^a donor H-bonds per molecule in the crystals;^b crystallographic data from Refs [8, 9]^c in the asymmetric unit there are two molecules, crystallographically not equivalent;^d crystallographic data from Ref. [7];^e these data do not concern the fusion;^f each NALPA molecule shares four hydrogen bonds with two NALPA and two hydrogen bonds with two water molecules (so that only three bonds per molecule must be energetically accounted); the water stoichiometrically bound, in turn, shares three hydrogen bonds with three NALPA molecules so that only 1.5 bonds must be accounted;^g from Ref. [10];^h L.Abate, private communication;ⁱ another broad peak is present with a maximum at about 382 K and $\Delta_{\text{trans}}H = 0.3(0.1)\text{kJ}\cdot\text{mol}^{-1}$;^j from Ref. [5]

Heat and temperature calibrations were performed by using high purity Indium. Baselines obtained with empty crucible at the same conditions as the measurements. Baseline subtractions and integration peak areas, were performed using the manufacturer computer program, previously tested and checked. For the fusion, the relevant onset temperatures were given by the program and controlled on the registration plot to avoid artifacts.

Some differences, beyond the calculated statistical errors, were found between the present results and those obtained previously [5, 10, 11]. These can be attributed to instrumental differences (especially in the automatical evaluation of the onset temperature) and small differences in sample purity.

Results and discussion

The temperatures and enthalpies of fusion, together with the corresponding entropies of fusion, are reported in Table 1. The numbers of hydrogen-bond donors per molecule in the crystals and the crystal densities are also reported in the last two columns of Table 1.

The enthalpies of fusion decrease on going from NAGA to NAAA to NALA i.e. on increasing the molecular weights and side-chain dimensions. In comparison, the enthalpies of fusion for NAVA, NAIA and NAPA are somewhat higher. Similar trends are shown in the entropies of fusion. The first set of data can be rationalised by noting that the NALA molecules share in the crystal only four hydrogen bonds (so that for each molecule there is an energy contribution from two hydrogen bond bridges, see Table 1) whereas for NAGA and NAAA six hydrogen bonds are shared by each molecule in the respective crystals. In the NALA crystals the energy contribution from the third potential hydrogen bond is annulled because of the necessity of accommodating the bulky side chains [4]. Also the density of solid NAGA is about 10% higher than that of *L*-NAAA and 18% higher than that of *L*-NALA (1.32, 1.20, 1.12 g cm⁻³ respectively) and shorter distances for intermolecular contacts, so higher cohesive forces necessarily result. The inversion of the trend of the enthalpies of fusion from that expected (an increase with increasing molecular weight) is thus consistent with the crystal structures. Similarly, the trend in the entropy changes on fusion appears to be largely determined by the structures of the crystalline solids and not by any increase in the degrees of freedom of the molecules when they are in the fused state.

The NAGA molecules, which are involved in a three-dimensional network of hydrogen bonds, are more hindered than the *L*-NAAA molecules, which are linked in two-dimensional networks of hydrogen bonds (the crystal structure of *L*-NAAA shows an alternating sequence of hydrophobic and hydrophilic layers, extending approximately parallel to the crystallographic *b-c* plane). In the crys-

tals of *L*-NALA the molecules are also less constrained with respect to either NAGA or *L*-NAAA crystals.

L-NALA melting profiles indicate (see footnote of Table 1) a small peak at about 382 K. This was frequently observed with different commercial and purified samples. This peak disappears however after fusion and recrystallization of the sample, and seems to arise from an irreversible dehydration and/or a solid-solid transition involving small amounts of the sample, rather than from impurities.

For the *L*-NAPA, in which only four hydrogen bonds per molecule are shared, the enthalpy of fusion is higher than that of *L*-NALA and lower than that of *L*-NAVA. In this instance, the reduced number of hydrogen bonds per molecule justifies the difference with *L*-NAVA. The higher packing density is probably responsible for the inversion (with respect to *L*- of *D*-NALA) of the expected dependence on molecular weight. The high packing density could be also responsible for the NAPA molecules behaviour, which in the crystal are in a relatively hindered situation when compared to that of NALA. This leads to the higher value of the entropy of fusion enthalpy. On the other hand the $\Delta_f H$ for *L*-NAPA is still higher than those of *L*-NAAA and NAGA for the higher molecular weight at comparable density of packing.

For *L*-NAVA and *L*-NAIA the enthalpies of fusion are much larger than those for the other N-acetylamides and a similar feature is found, at least for *L*-NAVA, in the enthalpies of sublimation [5]. Since, both *L*-NAVA and *L*-NAIA also have markedly higher entropies of fusion (70–80 against 40–60 J·K⁻¹·mol⁻¹ for the other N-acetylamides), it seems reasonable that, irrespective of the final state (vapour or melt) for both these substances, a further effect must be present during the fusion of the crystalline phases. This effect could arise from the population of numerous conformational states in the melts, which are not present in the crystals. In particular, it is likely that, both in the vapour and in the melt, steric hindrance, stemming from branching in the β position of the side-chains, allows to populate the high energy internal rotations, about the C ^{α} -C ^{β} bond, which are absent for the γ -branched enantiomers of NALA.

The two crystallized racemic mixtures behave in a very different manner. For *D*, *L*-NAAA the fusion temperature is only little lower than that of the pure enantiomer. Since the number of hydrogen bonds per molecule is the same in both the racemic and *L*-enantiomeric crystals, and in spite of the little lower density of the raceme, it seems that the formation of the racemic crystal leads not only to a gain of symmetry, but also to a small energetic gain from intramolecular contacts.

In contrast, for *D*, *L*-NALA both the enthalpy and entropy of fusion are an order of magnitude lower than those of both the pure enantiomers. This is so in spite of the crystallographic evidence which shows an increased number of hydrogen bonds in the racemic crystals. The lower density of these substances cannot account for the remarkable difference observed and since there is no evidence for any solid-solid transition from 250 K up to the temperature of fusion

of the crystals (404 K), we suggest that the low values of both $\Delta_f H$ and $\Delta_f S$, can arise from significant association of pairs of enantiomers in the fused state. It should be noted that the fused NALPA will be less polar than the fused NAAA, and consequently one would expect more intense polar interactions in the former.

When we come to consider the four dipeptide derivatives, a separate discussion is required for the NALPA-H₂O case. The low transition temperature, found for the NALPA-H₂O, arises from the dehydration of the crystals and not from fusion process. No other transitions were found up to 570 K, which is the temperature of decomposition of NALPA, nor traces of the first transition in successive scanning runs on the same sample, which became amorphous. Really a quite reproducible but irreversible dehydration process occurs near the boiling point of the water (in the NALPA crystals one water molecule is involved in three H-bonds). The transition enthalpy change ($\Delta_{\text{trans}}H$) observed, however, is more consistent with the value expected from the vaporization of one molecule of water, than from a transition from a crystalline to an amorphous state of the dehydrated peptide. The large value ($\sim 162 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$) of the ratio $\Delta_{\text{trans}}H/T_{\text{av}}$ indicates that the transition is not a first order process. It is worthy to note that the water molecules in the crystal originate from impurities of the solvent used in the synthesis and successive manipulations and are not easily removed during crystallizations from anhydrous solvents. Work is in progress to obtain samples of crystalline anhydrous peptide.

The enthalpy of fusion of NAA₂A is about twice that for NAAA, but this seems to be due to a fortuitous balance of different effects. During the fusion of the dipeptide, four H-bonds per molecule can be broken, against three in the case of NAAA crystals. However the packing of the dimer seems more effective, as indicated by the densities (1.244 for NAA₂A against 1.203 g cm^{-3} for NAAA). The entropy of fusion, as expected, is less than twice that of the monomer, owing to the relatively minor gain in degrees of freedom for the fused dipeptide. As a consequence a much higher melting temperature of the dipeptide results. It is possible that the same considerations could be applied for NAGAA, and that a comparison of the sum of enthalpies and entropies of fusion of NAGA and L-NAAA would be useful. Unfortunately, for both of these the melts start to decompose just after the end of fusion process, and so some uncertainties are present in the thermodynamic parameters.

For NAPGA the thermodynamic quantities are rather similar to those of both L-NAPA and NAGA and it seems that the presence of tetrahydropyrrolidine ring does not significantly perturb the stability of the glycol residues (and vice versa), with the result that the properties of NAPGA are similar to those of the constituent monomeric N-acetyl amides.

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Zusammenfassung — Mittels DSC wurden Schmelz- und Umwandlungsenthalpien und -temperaturen für vier substituierte Dipeptide (N-acetylamide von Glycyl-*L*-Alanin (NAGAA), *L*-Alanyl-*L*-Alanin (NAA₂A), *L*-Prolyl-Glycin (NAPGA) und *L*-Leucyl-*L*-Prolin-Monohydrat (NALPAH₂O)) bestimmt und die Schmelzentropien ermittelt. Die erhaltenen Ergebnisse wurden mit denen der entsprechenden substituierten Aminosäuren und einiger ihrer kristallinen Razemate verglichen. Die Schmelzenthalpien und -entropien einiger substituiertes Aminosäuren wurden wiederbestimmt. Die Ergebnisse werden im Vergleich mit den Kristallstrukturdaten diskutiert, die teilweise in der Literatur beschrieben sind oder von einigen der Autoren unlängst ermittelt wurden. Hauptsächlich auf der Grundlage der Anzahl intramolekularer Wasserstoffbindungen und den Packungsdichten innerhalb des Kristalles wurde eine rationelle Betrachtung der Schmelzparameter versucht.