Journal of Thermal Analysis and Calorimetry, Vol. 57 (1999) 119-132

CORRELATION BETWEEN PHASE TRANSITION THERMODYNAMICS AND CRYSTAL FEATURES OF SOLID SMALL PEPTIDES

G. Barone[1] *and R. Puliti*[2]

[1]Dipartimento di Chimica dell'Università 'Federico II', via Mezzocannone 4, 80134 Napoli
[2]Istituto per la Chimica di Molecole di Interesse Biologico* (CNR), via Toiano 6, 80072 Arco Felice, Napoli, Italy

Abstract

In this paper a review of recent results concerning thermodynamic properties of solid uncharged derivatives of some amino acids and small peptides is reported. The experimental data obtained by different calorimetric methods are: sublimation enthalpies, heat capacities, enthalpies and temperatures of fusion and, in few cases, enthalpies and temperatures of solid-to-solid transitions. The standard molar and specific enthalpies and entropies of sublimation at 298.15 K have been calculated integrating the heat capacities of solids and vapours as function of temperature or directly measuring by calorimetry the heating enthalpies. The first ones have been obtained by interpolation of the values calculated according to the group additivity method of Benson. The sublimation thermodynamic properties have regarded N-acetylamides of glycine (NAGA), *L*-alanine (*L*-NAAA), *L*-valine (*L*-NAVA), *D*- and *L*-leucine (*D*-NALA and *L*-NALA, respectively) and *L*-isoleucine (*L*-NAIA) as well as the cyclic dipeptides glycyl-glycine (*c*-Gly-Gly), glycyl-*L*-alanine (*c*-Gly-*L*-Ala), *L*-alanyl-*L*-alanine (*c*-*L*-Ala-*L*-Ala) and sarcosyl-sarcosine (*c*-Sar-Sar). Heat capacities of the solid phases have been included also for N-acetylamide of *L*-proline (*L*-NAPA), N'-methyl derivatives of the N-acetylamides previously cited and other amino acids, such as phenylalanine (F), isobutyric acid (isoBu), norvaline (norV) and norleucine (norL). In the text these substances are indicated as NAFAMe, etc. The heat capacities of their racemes are also reported. The fusion properties have concerned only two raceme mixtures (*D*,*L*-NAAA and *D*,*L*-NALA) and N-acetylamides of the cited amino acids, sarcosine (NASarA) and the following di- or tripeptides: glycyl-*L*-alanine (NAGAA), *L*-alanyl-*L*-alanine (NAA_2A), glycyl-*L*-proline (NAGPA), *L*-prolyl-glycine (NAPGA), *L*-leucyl-*L*-proline (NALPA) and *L*-prolyl-*L*-leucyl-glycine (NAPLGA). Finally, solid-to-solid transitions have been found and characterized for *L*-NALA and NAGPA. All thermodynamic properties are discussed in the light of the crystal packing parameters determined during parallel crystallographic studies. It allows a comprehensive rationale of the behaviour of the solid state and its transitions for this interesting family of substances.

Keywords: crystal packing, enthalpies and entropies of sublimation and fusion, solid heat capacities, solid uncharged peptides

Introduction

In this paper we want to review the results, obtained over the years in different laboratories, concerning thermodynamic properties of solid small peptides and other

* Associated to National Institute for the Chemistry of Biological Systems (CNR)

1418–2874/99/ $ 5.00

Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

correlated substances used as low molecular mass models for polypeptidic chains. These researches are of interest for a deeper understanding of the solid state physicochemical properties of polar organic substances bearing hydrogen bond donor and acceptor groups. It is also of interest to clarify the directional forces determining the unique physiological conformation of globular proteins, enzymes, carriers and regulation and restriction proteins being the more outstanding families. Systematic and coordinated thermodynamic and crystallographic researches, developed at laboratories of the authors, have recently permitted to rationalize some properties of solid N-acetylamides of several amino acids or small peptides. The flexibility of the side chains gives rise to a variety of crystal packings ruled mainly by effective assessments of H-bonds in infinite networks and by contemporary optimization of van der Waals contacts and accommodation of bulky side-chains. These factors determine the solid state properties (heat capacities, heating enthalpies) and transition parameters (enthalpies of sublimation and fusion) that depend on the cohesion energies. The rationalization of other thermodynamic transition properties (T_{trs}, $\Delta_{trs}S$) characterizing the overall stability of crystals, have required more detailed discussions about the freedom degrees gained by molecules passing from a particularly assembled organization in the molecular crystals to fluid phases (vapours, melts). More complex explanations are required for rationalizing the experimental results of some fusion processes taking into account a possible residual aggregation in the melt. Finally, it must be noted that a reliable assessment of thermodynamic derived properties can be tried only when they are exclusively obtained by combination of calorimetric results.

Sublimation thermodynamics

First of all we want to consider the sublimation thermodynamic properties of a group of N-acetylamides of simple amino acids that recently have been accurately redetermined [1]. These properties can be successfully rationalized on the basis of the crystallographic data, as we will discuss in the following. The set of substances can be described by the general formula CH_3–CO–[NH–CH(–R)–CO]–NH_2 where –*R* represents the side chain of a generic amino acid residue: –H for glycine, –CH_3 for alanine, –$CH(CH_3)_2$ for valine, –$CH_2CH(CH_3)_2$ for leucine, –$CH(CH_3)CH_2CH_3$ for isoleucine, for instance. These uncharged molecules seem to be useful models for studying some of the interactions occurring in globular proteins [2–4]. They were for the first time synthetized and proposed as models by Lilley and coworkers in a group of papers [5–7 and references quoted therein].

For obtaining sublimation enthalpies and entropies of some of these substances we have used, in preliminary works [8, 9], an indirect method based on the determination of temperature dependence of the vapour pressures measured by means of a Knudsen torsion-effusion apparatus [10–14]. The results were obtained exploring temperature ranges of 25÷50 degrees around 400 K, owing to reduced vapour pressures of the substances [8, 9]. The standard molar enthalpies and entropies at 298.15 K were then obtained by integration of the heat capacities of both vapours and solids [15]. The first ones were calculated as function of temperature according to the group contribution approach of Benson [16] and the second ones utilizing a

preliminary set of DSC measurements [15]. However, more recent and accurate calorimetric measurements of sublimation enthalpies [1] have only partially confirmed the results from the indirect method. Besides, a much more complete set of data, concerning the dependence on temperature of the heat capacities of solid N-acetylamides of amino acids and other correlated substances [17], allows to evaluate more reliable values for both standard enthalpies and entropies at 298.15 K [1].

The molar sublimation enthalpies $\Delta_{sub}H_m$ were obtained using a Tian-Calvet calorimeter, equipped with effusion Knudsen cells, working in the temperature range 298÷500 K and vapour pressure range 0.0133÷13.3 Pa [18, 19]. The enthalpy changes necessary to heat a mole of substance, from the standard to the measurement temperatures, were obtained for two compounds (*L*-NAIA and *L*-NALA) using too a Tian-Calvet calorimeter equipped with an oven (where the cell was equilibrated at the same temperature of sublimation experiments) and measuring the heat flux when the cell falls from the oven into calorimeter at 298.15 K. These results are in very good agreement with those obtained by integrating molar heat capacities of the crystalline solids, measured in turn with a Mettler DSC20 calorimeter [17].

Molar sublimation and heating enthalpies of a set of cyclopeptides were obtained by the same calorimetric methods [20]. The standard molar entropies at 298.15 K were also calculated by integration of experimental heat capacities of solids [17] and vapours, as before reported.

Crystal structures and sublimation properties

N-acetylamides of amino acids

The molar sublimation enthalpies and entropies at 298.15 K are reported in Table 1 together with crystal densities D_c and number of intermolecular hydrogen bonds $N_{H\text{-}bonds}$ per molecule in the crystal (from the energetical point of view, only half the energy involved in one H-bond pertains to each molecule) [9, 21, 22]. Other important crystallographic parameters, whose discussion is beyond the limits of this review, are reported in the quoted references from our laboratories. The packing parameters are strictly dependent on bulkiness of the residue side chains and on possibility to accommodate their, more or less easily, without perturbing the overall crystal stability. *L*-NAVA, *L*-NALA and *L*-NAIA molecules, f.e., assemble in the crystals in such a way to form layers containing alternately or networks of H-bonds or hydrophobic groups only, so the structures are characterized by cleavage planes [9, 21, 22]. Moreover, in the *L*-NALA crystal, for accommodating the extended side chain, the sheets of molecules interconnected by H-bonds are wrinkled up to assume a half egg box shape [21]. In this structure only four hydrogen bonds, of the six possible, are shared by each molecule. Vice versa in the NAGA crystal, the absence of alkyl side chains allows a more compact packing characterized by a tridimensional pattern of hydrogen bonds, so that short and effective intermolecular contacts are established and the D_c results 10–17% higher than those of the other substances. Consequently, a regular trend cannot be evidenced for the standard molar properties. A rationalization can be instead attempted considering specific sublimation enthalpies (kJ g^{-1} instead of kJ mol^{-1}) reported in the fifth column of Table 1. It appears that the

Table 1 Molar and specific sublimation enthalpies and entropies at 298.15 K of some N-acetylamides of amino acids and some cyclic dipeptides *vs*. molecular masses (M_m), number of hydrogen bonds pertaining to each molecule in the crystal ($N_{H\text{-}bonds}$) and crystal densities (D_c)

Substances	$\Delta_{sub}H^0_m$ / kJ mol^{-1}	$T\Delta_{sub}S^0_m$ / kJ mol^{-1}	M_m g mol^{-1}	$\Delta_{sub}H^0_{sp}$ / kJ g^{-1}	$T\Delta_{sub}S^0_{sp}$ / kJ g^{-1}	$N_{H-bonds}$	D_c/ g cm^{-3}
NAGA	126.3(2.3)	67.3	116.120	1.088	0.583	3	1.319
L-NAAA	118.1(1.6)	63.2	130.147	0.907	0.486	3	1.203
L-NAVA	133.1(2.2)	67.4	158.200	0.841	0.426	3	1.135
L-NALA	119.8(1.5) 120.8(1.6)[a]	63.5	172.227	0.696 0.701	0.369	2	1.125
D-NALA	120.4(0.4)[b,c]	–	172.227	0.699	–	–	–
L-NAIA	147.5(0.3)[b] 147.4(0.4)[a,b]	73.3	172.227	0.856 0.856	0.426	3	1.165
c-Gly-Gly	118.7(0.3) 118.5(0.3)[a]	47.1	114.104	1.040 1.038	0.413	2	1.592
c-Gly-*L*-Ala	143.1(1.4) 145.3(1.4)[a]	69.6	128.131	1.117 1.134	0.543	2	1.421
c-*L*-Ala-*L*-Ala	137.9(0.3) 137.4(0.3)[a]	63.4	142.158	0.970 0.966	0.446	2	1.312
c-Sar-Sar	92.6(0.3) 93.0(0.3)[a]	48.9	142.158	0.647 0.654	0.344	0	1.371

From Refs [1, 9, 20–29]. In parentheses are reported the standard deviations from the average experimental data. Temperatures of sublimation were 376.15 K for the first four substances; *T*=390.15 K for *L*-NAIA, *c*-Gly-*L*-Ala and *c*-*L*-Ala-*L*-Ala; *T*=393.15 K for *D*-NALA; *T*=410.15 K for *c*-Gly-Gly; *T*=360.15 K for *c*-Sar-Sar

[a] Values obtained by direct heating calorimetry,

[b] From only two sublimation measurements,

[c] Values obtained using the molar heat capacities of *L*-NALA for integrating from 298.15 to 393.15 K and adding 0.3 kJ mol^{-1} more for the $\Delta_{trs}H$ at 382 K (see text)

values of specific enthalpies decrease regularly from NAGA to *L*-NAVA, likewise the crystal densities, the *L*-NALA sublimation enthalpy resulting much lower in consideration of the reduced number of hydrogen bonds per molecule. The specific sublimation enthalpies then suggest a general decrease in the cohesion energies of the crystals at decreasing in the packing efficiencies for the bulkiness of alkyl side chains. However that is only a preliminary rationale. The sublimation process, really, requires energy to activate and differently populate, not only the translational but also vibrational and overall and internal rotational levels. This explains the very large values of $\Delta_{sub}H^o_m$, if compared with the energy required for breaking three (two for *L*-NALA) H-bonds and van der Waals interactions. Hence, the standard molar enthalpies are the result of conflicting contributions at increasing the molecular masses: from one hand less energy is required for destroying the crystal lattice of

larger molecules, on the other, more energy is required for populating all the accessible states in the gas phase at 298.15 K.

For what concerns sublimation entropies, it must be considered that these parameters are determined not only by the high number of overall and internal freedom degrees gained in the gas phase by the more complex molecules against the more simple ones, but also by the constrains limiting molecular motions especially in the more compact crystals. From this point of view, the presence in NAGA crystals of a tridimensional H-bond pattern undoubtedly contribute to the very high values of $\Delta_{sub}S^{o}_{m}$ and corresponding specific parameter, as the librational and vibrational modes are much more hindered in this solid than in *L*-NAAA and *L*-NALA. Besides, in the last case, the reduced number of hydrogen bonds allows more freedom to the overall molecule and its frameworks in the crystal lattice. The very high values of both specific and molar sublimation entropies of *L*-NAIA and *L*-NAVA with respect to *L*-NALA have the same explanation before discussed. The molar entropies of *L*-NAIA and *L*-NAVA are also the highest among those here reported, probably because their compact side chains (β-branched) gain much more freedom against those of *L*-NAAA and NAGA passing to the vapour phase. Actually other contributions due to the distribution of populations on translational, vibrational and rotational modes in the gas phase are very important in determining the sublimation entropy values. Finally, all the contributions due to different freedom degrees in the crystals and vapours lead to a trend of the specific sublimation entropies opposite to that of molecular masses. This trend is quite similar to that of the specific enthalpies with an only small inversion between the *L*-NAVA and *L*-NAIA values actually differing for less than 2%.

Cyclic dipeptides

Standard molar sublimation enthalpies and entropies concerning four cyclic dipeptides (*c*-Gly-Gly, *c*-Gly-*L*-Ala, *c*-*L*-Ala-*L*-Ala and *c*-Sar-Sar) [20] are also reported in Table 1. The experimental calorimetric data were obtained, as for N-acetylamides, at relatively high temperatures given for each substance in footnote of the Table. The standard molar quantities are obtained taking into account the enthalpies necessary for heating up the solids from 298.15 K to the temperatures of measurements. These enthalpies were obtained as previously described and are in good agreement with those from integration of $C^{o}_{p,m}(s)$ [17]. The enthalpies for cooling the vapours were instead obtained by integration of the molar heat capacities evaluated in the same temperature range using the Benson method [16]. The molar sublimation enthalpies are comparable, as order of magnitude, with those of the N-acetylamides, except for *c*-Sar-Sar which is very low. The sublimation enthalpies can be correlated with the number of intermolecular hydrogen bonds and the crystal densities [23–29]. With respect to N-acetylamides, the cyclo-dipeptides show a higher packing efficiency, due to flatness of the molecules that permits good contacts among the H-bonded ribbons. That compensates the reduced number of H-bonds and contributes to the crystal stability. In particular, the drastically reduced enthalpy value of *c*-Sar-Sar is undoubtedly due to absence of H-bonds in the crystal [23]: in the other three diketopiperazine structures four H-bonds are shared by each molecule [24–29]. At this moment the relatively high value of $\Delta_{sub}H_{sp}$ of *c*-Gly-*L*-Ala is difficult to ex-

plain. lt could be attributed to a particular distribution of matter in the crystal that permits efficient intermolecular contacts, apart from what can be immediately inferred from the D_c value. The same explanation could rationalize the relatively high value of the specific sublimation entropy. This working hypothesis (or other alternative explanations) will be better checked when detailed structural information will be available.

Heat capacities and solid-to-solid transitions

Accurate measurements of molar heat capacities for NAGA and four *L*-amino acid derivatives have been reported in a recent paper [17], together with those of other related substances, such as the N-acetylamide of *L*-proline and the four cyclic dipeptides before considered. The results have been compared with those of N-acetyl N′-methylamides of amino acids reported in a previous paper [30]. All these data are summarized in Table 2. The main conclusion of the authors outlines the substantial additivity of CH_2 contributions to the molar heat capacities at 298.15 K of these and other solid organic compounds. The contribution of each CH_2 group to $C^{o}_{p,m}(s)$ was estimated 26±1 J K^{-1} mol^{-1} for N-acetylamides and cyclopeptides [17], *vs.* 25±1 J K^{-1} mol^{-1} for amino acids [31–33], N′-methylated amides and their racemes [30] and 24 J K^{-1} mol^{-1} for alkanes [34]. Only exceptions were *L*-proline and derivatives, the values of which were appreciable lower than those of *L*-Val and derivatives, while the molecular masses differ two daltons only. These discrepancies have been attributed to the presence of the pyrrolidine ring. The analogous behaviour of N-acetyl N′-methylamide of *L*-phenylalanine was likewise attributed to the presence of the phenyl group. Therefore, the CH_2 additivity seems to be a constant characteristic of many solid organic compounds [35]. Looking at the known crystal structures of these substances [36–46], the observed CH_2 additivity can be well attributed to a proportional amount of freedom gained by the alkyl chains at increasing temperature near the standard value of 298.15 K, with the obvious exception of more hindered proline and phenyl rings. The small differences observed among the $C^{o}_{p,m}(s)$ and $dC_{p,m}(s)/dT$ values of the isomers of Val, Leu and derivatives, can be attributable to a more or less compact arrangement of the respective side chains in the crystals. Indeed, the gain of mobility of alkyl groups during the heating require different amounts of energy.

No difference, for instance, in the $C^{o}_{p,m}(s)$ values of *L*-NALA and *L*-NAIA appears at 298.15 K. However the $dC_{p,m}(s)/dT$ of *L*-NALA is about 18% higher that of *L*-NAIA. This fact can be attributed to the higher freedom of leucine side chain and other frameworks not anchored by H-bonds, with respect to the corresponding parts of *L*-NAIA [21, 22]. The hypothesis is confirmed by the existence for *L*-NALA of a solid-to-solid transition observed at 382 K (temperature intermediate between the sublimation, $C^{o}_{p,m}(s)$ determination and fusion temperatures) [47]. This transition, characterized by a small value of $\Delta_{trs}H = 0.3\pm0.1$ kJ mol^{-1}, can be attributed to a disordering process of the leucine side chain, scarcely cooperative in the crystal. This phenomenon is irreversible and the calorimetric peak of the transition disappears in successive cycles of cooling and heating or after fusion.

Similarly, the difference of about 14% between the $dC_{p,m}(s)/dT$ values of *c*-*L*-Ala-*L*-Ala and isomeric *c*-Sar-Sar can be undoubtedly attributed to the different

structures of the two crystals [23, 26–28]. The cyclo-sarcosyl-sarcosine molecules, as discussed in the previous section, are more free to librate and oscillate in the crystal for the lacking of hydrogen bonds, instead present in the *L*-alanyl-*L*-alanine and other cyclopeptide crystals.

Table 2 Standard molar heat capacities $C^{o}_{p,m}(s)$ of some N-acetylamides of amino acid and some cyclic peptides at 298.15 K with the 95% confidence limits in parentheses and corresponding temperature derivatives $dC_{p,m}(s)/dT$ in the given ranges

Substances	$C^{o}_{p,m}(s)$/ J K^{-1} mol^{-1}	$dC_{p,m}(s)/dT$/ J mol^{-1}	T_{range}/ K
NAGA	152.7(0.8)	0.5645	318÷378
L-NAAA	181.3(0.5)	0.6066	318÷383
L-NAPA	208.9(0.8)	0.7509	318÷378
L-NAVA	232.1(0.6)	0.6435	218÷403
L-NALA	259.6(1.8)	0.8720	318÷368
L-NAIA	261.9(0.7)	0.7494	318÷388
NAGAMe	178.0(2.5)	0.6562	318÷373
L-NAAAMe	201.3(0.8)	0.5510	318÷373
L-NAPAMe	221.3(1.6)	1.0931	319÷343
L-NAVAMe	245.1(1.0)	0.7329	318÷403
L-NALAMe	281.5(1.4)	0.8275	318÷378
L-NAIAMe	272.5(1.4)	0.7471	318÷373
L-NAFAMe	277.1(2.3)	0.7595	318÷398
D,*L*-NAAAMe	196.2(1.1)	0.6223	318÷373
D,*L*-NAisoBuAMe	228.9(1.3)	0.7052	318÷373
D,*L*-NAPAMe	233.0(1.2)	0.8524	319÷343
D,*L*-NAVAMe	256.6(1.3)	0.6814	318÷408
D,*L*-NAnorVAMe	237.4(2.6)	0.9384	318÷373
D,*L*-NALAMe	271.5(1.3)	1.0409	318÷373
D,*L*-NAIAMe	269.2(1.1)	0.7937	318÷373
D,*L*-NAnorLAMe	287.6(1.8)	0.7716	318÷383
D,*L*-NAFAMe	277.9(1.6)	0.9018	318÷403
c-Gly-Gly	135.7(0.3)	0.3688	318÷413
c-Gly-*L*-Ala	161.4(0.4)	0.4383	318÷413
c-*L*-Ala-*L*-Ala	191.4(0.4)	0.4914	318÷413
c-Sar-Sar	186.5(0.9)	0.5633	318÷363

From Refs [17, 30]

Another pre-melting thermal phenomenon was found in the case of N-acetyl-glycyl-*L*-prolinamide [48]. The enthalpy and entropy values (5.6 kJ mol^{-1} and 12–13 J K^{-1} mol^{-1}, respectively) for this transition were evaluated from the deconvolution of the calorimetric traces (Fig. 1). These remarkable values quite correspond to the differences between the fusion enthalpies and entropies of this compound and its isomer N-acetyl-*L*-prolyl-glycinamide (27.9±0.4 *vs.* 32.2±0.4 kJ mol^{-1} and 59.0±0.9 *vs.* 73.7±0.8 J K^{-1} mol^{-1} respectively, Table 3). So, we assumed that a solid-to-solid transition occurs. The values of the transition parameters (about 20% of the total fusion enthalpy) led to try a more deep structural interpretation. Actually no change in optical properties of the crystals were observed before the fusion, nor conformational changes [49] in the proline ring alone can justify the value of $\Delta_{trs}H$. Looking at the X-ray results, it can be inferred that slight conformational adjustments of part of the molecule can disturb the local crystal symmetry without altering the overall crystalline habitus. For instance, small conformational changes in the glycine residue can be sufficient to lose the 2_1 symmetry along the *a* direction, moving from orthorhombic to monoclinic space group with lower symmetry. Two independent molecules, with slight conformational differences alone, would form the new asymmetric unit in the crystal, probably involving the break of one hydrogen bond per pair of molecules, that can justify the enthalpy value. The consequent statistical disorder makes the pre-melting process irreversible [48]. When other scanning runs are carried after cooling of the sample only the melting process was revealed, the onset temperature T_f and the $\Delta_f H$ being coincident with the values obtained from the deconvolution of the formerly registered scan. Only when the samples are fresh obtained by repeating crystallization procedure both the processes were correctly reproduced.

Fusion thermodynamics and crystal packing

Enthalpies, entropies and temperatures of fusion of 20 derivatives of simple amino acids and peptides are reported in Table 3, together with the number of hydrogen bonds pertaining to each molecule and crystal densities. Interesting aspects can be discussed by grouping the substances according to a rationale that will become evident in the following. We will compare the thermodynamic stability of crystals considering mainly the significant quantities $\Delta_f H_m$ and $\Delta_f S_m$. Then a first set of substances to be discussed is formed by NAGA, *L*-NAAA and N-acetylamides of sarcosine (NASarA) and of *L*-proline (*L*-NAPA), the second by *D*-NALA, *L*-NALA, *L*-NAVA and *L*-NAIA. Considering the first four substances, it has been focused that NASarA properties [22] are quite similar to that of NAGA instead of the isomeric *L*-NAAA, in spite of the fact that NASarA molecules are linked in the crystal to neighbours by only two independent hydrogen bonds, *vs.* the three found in the other two structures. The same behaviour is shown by *L*-NAPA [17, 47]. This fact can be only in part attributed to the more efficacious packings of NASarA [22] and *L*-NAPA [38, 39] (D_c values in Table 3). Moreover, at least in the case of *L*-NAPA, the higher values of $C^o_{p,m}(s)$ and $dC_{p,m}/dT$ suggest an increasing internal mobility of the molecules due to a weakening of the intermolecular interactions in the crystal at inereasing temperature. A more complete rationale must be therefore searched in the properties of the final states, i.e. the melts. A reliable explanation is that in the liquid phases of

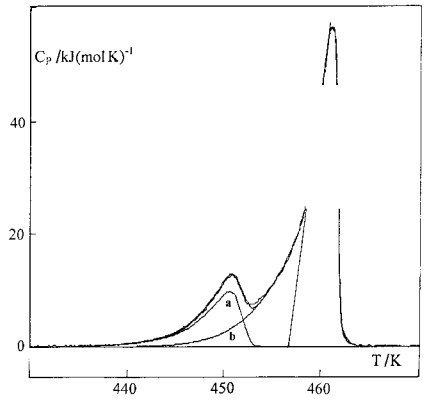

Fig. 1 Experimental calorimetric profile of NAGPA, corrected for the calibration and baseline subtraction, with the two peaks (*a* and *b* curves) obtained by deconvolution and their recalculated convolute curve superimposed. The onset temperature was calculated by extrapolation carried on the curve *b* (straight line). In successive runs the first peak disappears and the experimental curve (not reported) practically coincides with the deconvoluted curve *b* of the first run (Redrawn from Ref. [48])

NASarA and *L*-NAPA the intermolecular interactions are weaker and more unstable than for *L*-NAAA, since the residual short living interactions (H-bonds, dipolar interactions, dispersive forces) occur in the last substance more likely or work more effectively. Then more energy must be provided and more freedom is gained by NASarA and *L*-NAPA with respect to *L*-NAAA during the phase transition. The $\Delta_f H$ and $\Delta_f S$ values become hence comparable with those concerning NAGA whose crystal are made stable by a tridimensional network of H-bonds and crowding of the molecules. Unfortunately, the knowledge of the melt properties is very poor, nor sufficient data concerning liquid amides are at present available.

In the second group of four N-acetylamides, the fusion thermodynamic properties of *L*-NAVA and *L*-NAIA (with β-branched side chains) are much higher than those of two enantiomers *D*- and *L*-NALA. The higher T_f values of *L*-NAVA and *L*-NAIA are due to the enthalpic terms, rather than entropic ones (Table 3). In fact, in the fusion process it needs to break more hydrogen bonds than in *D*- and *L*-NALA. Further energetic contributions are required for activating the hindered rotational states around C^{α}–C^{β} bonds of *L*-NAVA and *L*-NAIA. These states require more energy to be populated with respect to those of γ-branched chain of *L*-NALA. The $\Delta_f S_m$ differences among these two pairs of acetylamides are still large but less remarkable than the differences among the $\Delta_f H_m$. The more compact shapes of the β-branched chains of *L*-NAVA and *L*-NAIA permit to the overall molecules to rotate more freely in the melt, while the more extended side chain of NALA easily promotes entanglements with neighbour molecules that interfere with the respective free rotations.

Because of the compensating effect previously discussed, the higher homologues of the N-acetylamide series show, for the fusion thermodynamic properties, a roughly increasing trend, in spite of the higher densities of the lower terms. An additive behaviour cannot be evidenced, contrarily to that observed for the heat capacities. Actually, for

the fusion properties an important role is undoubtedly played by the interactions in liquid phase that prevent a regular trend even for the specific properties (not reported).

That the residual interactions in the liquid phase could play, in some cases, an important role can be emphasized by comparing the melting properties of the crystals of NAAA and NALA racemes [50]. For *D,L*-NAAA in fact the fusion temperature

Table 3 Temperature, enthalpies and entropies of fusion, together with crystal packing parameters of some N-acetylamides of amino acids and some cyclic dipeptides

	T_f/ K	$\Delta_f H_m$ / kJ mol^{-1}	$\Delta_f S_m$ / J mol^{-1} K^{-1}	$N_{H-bonds}$	D_c/ g cm^{-3}
NAGA	410.9(1)	27.0(1)	65.7(3)	3	1.319
NaSarA	412.7(1)	27.4(3)	66.4(7)	2	1.276
L-NAAA	435.4(1)	23.7(3)	54.4(7)	3	1.203
L-NAPA	418.7(1)	28.1(1)	67.1(2)	2	1.294
L-NALA[a]	401.4(3)	17.4(3)	43.3(3)	2	1.125
D-NALA	401.4(1)	16.2(3)	40.3(7)	–	–
L-NAVA	509.0(2)	36.9(4)	72.5(8)	3	1.135
L-NAIA	529.6(2)	41.8(1)	78.9(2)	3	1.165
D,L-NAAA	433.6(3)	25.3(1)	58.3(3)	3	1.168
D,L-NALA	401.0(2)	2.8(1)	7.0(2)	3	1.077
NAGAA	522.7(2)	50.6(5)	96.8(10)	4	1.340
NAA_2A	511.2(4)	38.1(8)	74.5(16)	4	1.244
NAPGA	434.1(1)	32.2(4)	73.7(5)	3	1.342
NAGPA[b]	457.8(4)	27.0(4)	59.0(9)	3	1.331
NALPA·H_2O	349.0(2)[c]	61.5(4)[c]	–	3.5+1.5[d]	1.208
NAPLGA·0.5H_2O	438.6(3)	36.2(4)	82.5(9)	3.5+1+0.5[e]	1.167
c-Gly-Gly	~560(decomp.)	–	–	2	1.592
c-Gly-*L*-Ala	543.9(2)	30.6(2)	56.3(4)	2	1.421
c-*L*-Ala-*L*-Ala	556.1(3)	30.6(2)	55.1(4)	2	1.312
c-Sar-Sar	418.2(3)	22.0(3)	52.7(8)	0	1.371

From Refs [17, 22, 36–54]

[a] A pre-melting solid-to-solid thermal effect was found at 382 K (see footnote c in Table 1)

[b] A pre-melting solid-to-solid transition was found at 450.6 K (see Fig. 1)

[c] These data concern the probable dehydration of the crystals, not the fusion process

[d] Each NALPA molecule shares four hydrogen bonds with two other NALPA molecules and three H-bonds with three water molecules, so that 3.5 H-bonds per peptide molecule must be energetically accounted. Water molecule in turn shares three H-bonds with three peptide molecules, so that 1.5 H-bonds per water are accounted

[e] Three and half H-bonds are energetically computed per NAPLGA molecule (six H-bonds shared with three peptide molecules and one shared with a water molecule) plus one intramolecular. Half a H-bond perinent to the stoichiometric half water molecule

as expected is just a little lower than that of the pure *L*-enantiomer, while $\Delta_f H_m$ and $\Delta_f S_m$ result both a little higher. The last values have been attributed to the higher symmetry characterizing the packing of the raceme against the pure enantiomer. That allows more effective intermolecular contacts, in spite of the lower crystal density, and a more ordered molecular arrangement. The behavior of *D*,*L*-NALA vice versa is widely different. The T_f, very similar to that of the pure enantiomer, is actually determined by the $\Delta_f H_m$ and $\Delta_f S_m$ values, both an order of magnitude lower. As no solid-to-solid transitions were found from 250 K up to the fusion temperature, it was assumed that these very low values arise from the presence, in the liquid raceme mixture, of a significant association between pairs of enantiomers. The association can be promoted by H-bonds and/or dipolar interactions more intense in the less polar fused *D*,*L*-NALA with respect to fused *D*,*L*-NAAA [47, 50].

The fusion thermodynamics of the N-acetylamides of some di- and tripeptides have been partly discussed in previous works on the basis of crystal packing features [51–54]. Here we want to summarize the discussions because the results require an overall explanation. For instance, it results that the enthalpies of fusion of N-acetylglycyl-*L*-alaninamide (NAGAA) and N-acetyl-*L*-alanyl-*L*-alaninamide (NAA_2A) are about the sums of those for the respective monomers (NAGA and NAAA). Really, in both cases four hydrogen bonds must be broken during the fusion, against three for the monopeptide derivatives, but the higher densities and the much more numerous intermolecular van der Waals contacts occurring in the dipeptides are sufficient to justify the observed values. The fusion entropies, as expected, are much less than the sum of the values found for the monomeric compounds, owing to the not proportional gain in degrees of freedom in the fused dipeptides. As a consequence, much higher T_f values are shown by the two dipeptides. Some uncertainty, however, affects the reproducibility of the data, because both the melts start to decompose just after the end of fusion scansions [51].

Considering now the four compounds containing *L*-proline, other interesting features need to be discussed. One of the dipeptides (N-acetyl-*L*-leucyl-*L*-prolinamide: NALPA) crystallizes with one water molecule strongly linked through three shared hydrogen bonds to the surrounding peptide molecules. The values reported in Table 3 actually do not refer to the fusion or a solid-to-solid transition but rather to a dehydration process, as can be evidenced by the very large enthalpy value and very low temperature. The last is the temperature corresponding to the maximum of the thermal effect, not the onset temperature as evaluated for all the fusion processes determined by DSC method. No other thermal effects were observed up to the decomposition temperature (570 K). The dehydrated substance becomes amorphous and the thermal effect is irreversible, so a reliable value of $\Delta_{dehydr} S$ cannot be calculated [52]. The analysis of the solid after the thermal transition confirms the complete water removal.

On the other hand NAGPA undergoes, in turn, a pre-fusion transition involving 5.6 kJ mol^{-1} and an estimated entropy change of ~12.5 J mol^{-1} K^{-1}, but this transition corresponds to a limited preliminary destruction of the tridimensional ordered interactions in the crystal [48]. The sums of the transition and fusion enthalpies and entropies, in fact, give about the same values of $\Delta_f H_m$ and $\Delta_f S_m$ of the isomer NAPGA [53] having the identical number of intermolecular H-bonds in the crystal

(Table 3). Instead the $\Delta_f H_m$ of the half hydrated tripeptide NAPLGA [54] is not high enough, when compared with that of NAPGA, to be consistent with the breaking of all the hydrogen bonds. Actually one of these H-bonds is intramolecular and $\Delta_f H_m$ value gives a strong indication that it remains stable in the melt, as suggested also by the limited difference between the fusion entropies of NAPLGA and NAPGA [53, 54]. NMR results seem to support this conclusion [55]. Moreover the 4 kJ mol^{-1} of difference between the enthalpies of NAPLGA and NAPGA, as so as the 9 J mol^{-1} K^{-1} between the $\Delta_f S_m$ values, seem to exclude the vaporization of crystallization water during the fusion at 438.6 K. On the other hands an abrupt vaporization of water cannot occur below of T_f, otherwise a behaviour similar to that of NALPA monohydrate would be found. Therefore, the maintenance of the crystallization water, strongly linked to tripeptide molecules, must very little contribute to the fusion thermodynamics [54]. Actually one of the factors reducing the difference between the $\Delta_f H_m$ of NAPLGA and NAPGA is the less dense packing of tripeptide that limits the number of intermolecular interactions per molecule in the solid. In turn the gain of mobility of leucine side chain and the partial breaking of the H-bonds involving water, during the fusion, will be partially balanced by the maintenance of the intramolecular H-bond that prevents the opening of β-turn structure. Furthermore a complete dissociation of water from tripeptide in the melt would involve an entropic 'cratic' contribution of about 8÷10 J mol^{-1} K^{-1} by itself [56].

Applying a roughly additive approach it is possible to evaluate from the $\Delta_f H_m$ a mean value of the energy per H-bond, ε_{H-bond}, in all these crystalline substances. Assuming a value of 1.2±0.5 kJ mol^{-1} for the van der Waals contribution of an aliphatic hydrogen linked to a carbon atom, as evaluated from the $\Delta_f H_m$ of linear hydrocarbons [57], a mean value of 5.9±0.5 kJ mol^{-1} was obtained for ε_{H-bond} [58]. It is in agreement with the value of 5.4 kJ mol^{-1} estimated for the melting of α-helix of a C-50 peptide, alanine rich, synthetized on this purpose [59]. These values are referred to condensed phases and are obviously lower than the value calculated in vacuo for the H-bond energy of a peptide-peptide or peptide-water or water-water pair, which amounts to about 25 kJ mol^{-1} [60].

The crystal structures of dipeptides are discussed in detail in the quoted references; that of NAPLGA, together with the results of potential energy minimization, is discussed in the paper [54].

No extended comments are required for fusion properties of the cyclic dipeptides. In fact one of they (*c*-Gly-Gly) decomposes before melting at a relatively high temperature (~560 K). On the other hands *c*-Gly-*L*-Ala and *c*-*L*-Ala-*L*-Ala show very similar properties [17], at least at temperatures very far from those of the sublimation measurements. The low fusion temperature of *c*-Sar-Sar is clearly due to the lower value of $\Delta_f H_m$ (22.0 *vs*. 30.6 kJ mol^{-1}) attributable to absence of H-bonds in the crystal (instead, the value of $\Delta_f S_m$ is very near to those of other two dipeptides). The relatively high values of temperature, enthalpy and entropy of fusion, in comparison with those of the N-acetylamides of analogous mass, must be attributed to the efficient molecular interactions occurring in the cyclopeptide crystals (the high D_c values in Table 3).

Conclusions

The strict comparison of the thermodynamic data with the crystal structure information proved to be a very successful approach for rationalizing the solid and phase transition properties of this family of substances. It is worth nothing that the densities of globular proteins are comparable to that of these crystalline solids. The standard heat capacities of the substances here discussed show a plain group additivity at 298.15 K that is only partially maintained by temperature derivatives of $C_{p,m}(s)$ and sublimation properties. A preliminary rationale can be based on crystal packing parameters (number of hydrogen bonds and densities), as so as on the activation of freedom levels attained by the overall molecules and by the flexible side chains when the molecules pass in the gas phase. The features of solid-to-solid transitions and fusion processes have required more complex hypotheses. In these cases and when we will deal with solution processes, the experimental results force to develop more complex explanations that must take into account possible residual aggregations in the melts or preferential solvatations of groups of different polarity in diluted solutions. Other work is required to accurately compute the reticular energies for these substances whose crystals show different patterns of hydrogen bonds and side chain arrangements, besides normal intermolecular interactions stabilizing simple molecular crystals.

* * *

This research was financially supported by the Italian 'Consiglio Nazionale delle Ricerche' (CNR) and by the COFIN MURST 97 CFSIB project of the 'Ministero dell'Università e della Ricerca Scientifica e Tecnologica' (MURST), Rome.

References

1 P. Del Vecchio, G. Barone, R. Sabbah, G. Della Gatta and L. Abate, submitted to J. Chem. Thermod. (1998).
2 T. H. Lilley in M. Jones (Ed.), Biochemical Thermodynamics, Elsevier, Amsterdam 2nd ed. Chap. 1, 1988.
3 G. Barone and C. Giancola, Pure and Appl. Chem., 62 (1990) 57.
4 G. Barone, P. Del Vecchio and C. Giancola, J. Solution Chem., 21 (1992) 1093.
5 G. M. Blackburn, T. H. Lilley and E. Walmesley, J. Chem. Soc. Faraday Trans. I, 76 (1980) 915.
6 H. E. Kent, T. H. Lilley, P. J. Milburn, M. Bloemendal and G. Somsen, J. Solution Chem., 14 (1985) 101.
7 G. M. Blackburn, T. H. Lilley and P. J. Milburn, J. Chem. Soc., Faraday Trans. I, 82 (1986) 2965.
8 D. Ferro, G. Della Gatta and G. Barone, J. Thermal Anal., 34 (1988) 835.
9 R. Puliti, C. A. Mattia, G. Barone, G. Della Gatta and D. Ferro, Thermochim. Acta, 162 (1990) 229.
10 V. Piacente, P. Scardola, D. Ferro and R. Gigli, J. Chem. Eng. Data, 30 (1985) 372.
11 D. Ferro and V. Piacente, Thermochim. Acta, 90 (1985) 378.
12 D. Ferro, G. Barone, G. Della Gatta and V. Piacente, J. Chem. Thermodyn., 19 (1987) 915.
13 D. Ferro and G. Della Gatta, Thermochim. Acta, 122 (1987) 189.
14 G. Barone, G. Della Gatta, D. Ferro and V. Piacente, J. Chem. Soc., Faraday Trans. I, 86 (1990) 75.

15 L. Abate, G. Barone, P. Del Vecchio, G. Della Gatta, C. Giancola and R. Sabbah, Nuovo Cimento, Conference Proceedings, 43 (1993) 273.
16 J. W. Benson, in Thermodynamical Kinetics, Wiley, New York, Chap. 2 (1968).
17 L. Abate, B. Palecz, C. Giancola and G. Della Gatta, J. Chem. Thermodyn., 29 (1997) 359.
18 R. Sabbah, I. Antipine, M. Coten and L. Davy, Thermochim. Acta 115 (1987) 153.
19 R. Sabbah and L. El Watik, Can. J. Chem., 70 (1992) 24.
20 P. Del Vecchio, G. Della Gatta and G. Barone, to be submitted (1999).
21 R. Puliti, C. A. Mattia, G. Barone and C. Giancola, Acta Cryst., C45 (1989) 1554.
22 R. Puliti, C. De Sena and C. Giancola, J. Thermal Anal., 48 (1997) 1249.
23 R. Degeilh and R. E. Marsh, Acta Cryst., 12 (1959) 1007.
24 P. Groth, Acta Chem. Scand., 23 (1969) 3155.
25 T. Srikrishnan, P. K. S. Gupta and R. Parthasarathy, Am. Cryst. Assoc. Ser. 2,5 (1977) 34.
26 E. Benedetti, P. Corradini and C. Pedone, Biopolymers, 7 (1969) 751.
27 E. Benedetti, P. Corradini and C. Pedone, J. Phys. Chem., 73 (1969) 2891.
28 E. Sletten, J. Amer. Chem. Soc., 92 (1970) 172.
29 S. M. Habermann and K. P. Murphy, Protein Sci., 5 (1996) 1229.
30 L. Abate, G. Della Gatta and G. Somsen, Thermochim. Acta, 239 (1994) 7.
31 C. H. Spink and I. Wadso, J. Chem. Thermodyn., 7 (1975) 561.
32 J. O. Hutchens, A. G. Cole and J. W. Stout, J. Am. Chem. Soc., 82 (1960) 4813.
33 A. G. Cole, J. O. Hutchens and J. W. Stout, J. Phys. Chem. 67 (1963) 1852.
34 M. G. Broadhurst, J. Res. Natl. Bur. Stand. (U.S.), 67A (1963) 233.
35 E. S. Domalski and E. D. Hearing, J. Phys. Chem. Ref. Data, 22 (1993) 805.
36 E. Benedetti, in M. Goodman and J. Meienhofer (Eds.), Peptides, Proc. 5th American Peptide Symposium, John Wiley and Sons, New York 1977, pp. 257–273.
37 E. Benedetti, in B. Weinstein (Ed.), Chemistry and Biochemistry of Amino Acids, Peptides and Proteins, Vol. VI, Dekker, New York (1982) pp. 105–184.
38 U. Drück, W. Littke and P. Main, Acta Cryst., B35 (1979) 253.
39 E. Benedetti, A. Christensen, C. Gilson, W. Fuller and M. Goodmann, Biopolymers, 15 (1976) 2523.
40 F. Iwasaki, Acta Cryst., B30 (1974) 2503.
41 Y. Harada and Y. Iitaka, Acta Cryst., B30 (1974) 1452.
42 T. Matsuzami and Y. Iitaka, Acta Cryst., B27 (1971) 507.
43 Y. Harada and Y. Iitaka, Acta Cryst., B33 (1977) 247.
44 A. Aubry, Ph. D. Thesis, Université de Nancy I; (1976) C.N.R.S., n, A.O.12610.
45 T. Ichikawa and Y. Iitaka, Acta Cryst., B25 (1969) 1824.
46 Y. Harada and Y. Iitaka, Acta Cryst., B30 (1974) 726.
47 G. Barone, C. Giancola, T. H. Lilley, C. A. Mattia and R. Puliti, J. Thermal Anal., 38 (1992) 2771.
48 R. Puliti, G. Barone, C. Giancola and C. A. Mattia, J. Mol. Struct., 382 (1996) 197.
49 C. Grathwohl and K. Wüthrich, Biopolymers, 20 (1981) 2623.
50 R. Puliti, C. A. Mattia, G. Barone and C. Giancola, Acta Cryst., C47 (1991) 1658.
51 R. Puliti and C. A. Mattia, Acta Cryst., C51 (1995) 336.
52 R. Puliti, C. A. Mattia and T. H. Lilley, Acta Cryst., C48 (1992) 709.
53 R. Puliti, C. A. Mattia and T. H. Lilley, Acta Cryst., C49 (1993) 2173.
54 R. Puliti, C. A. Mattia, C. De Sena and G. Barone, J. Mol. Struct., 442 (1998) l.
55 R. Walter, I. Bernal and L. F. Johnson, in Chemistry and Biology of Peptides, Ann Arbor Science Publishers, Ann Arbor, Mich., 1972, pp. 131–135.
56 W. Kauzmann, in Adv. Protein Chem., Vol. 14, 1959, pp. 1–64.
57 A. Nicholls, K. A. Sharp and B. Honig, Proteins: Struc. Funct. Genet., 11 (1991) 281.
58 G. Graziano, F. Catanzano, P. Del Vecchio, C. Giancola and G. Barone, Gazz. Chim. Ital., 126 (1996) 559.
59 J. M. Scholtz, S. Marquess, R. L. Baldwin, E. Y. York, J. M. Stewart, M. Santoro and D. W. Bolen, Proc. Natl. Acad. Sci. USA., 88 (1991) 2854.
60 J. Hermans, H. J. C. Berendsen, W. F. van Gunsteren and J. P. M. Postma, Biopolymers, 23 (1984) 1513.