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DSC-INVESTIGATIONS OF 22 CRYSTALLINE NEUTRAL ALIPHATIC AMINO ACIDS IN THE TEMPERATURE RANGE 233 TO 423 K

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ABSTRACT

The 22 crystalline neutral aliphatic amino acids under study, namely the DL- and L-forms of α-amino-*n*-butyric acid (**1,2**), serine (**3,4**), cysteine (**5,6**), norvaline (**7,8**), valine (**9,10**), threonine (**11,12**), norleucine (**13,14**), leucine (**15,16**), isoleucine (L-form only: **17**), methionine (**18,19**), and cystine (**20,21**; and also the meso-form: **22**) show nine solid-state phase transitions between 233 and 423 K by DSC-analysis. The phase transitions of **2** at 356 K, **7** at 390 K, **8** at 273 K, **14** at 389 K, **18** at 380 K, and **19** at 393 K are described for the first time.

INTRODUCTION

Twenty amino acids are found in naturally occurring proteins. In peptides the amino acids are bound by the functional amino and carboxyl groups in –CO–NH–peptide bonds building a chain. The crystal structure of many neutral aliphatic amino acids was examined by X-ray crystallography. In the crystalline state the functional groups of neutral aliphatic amino acids are held together by hydrogen bonds. It is known that solid-state phase transitions exist in this kind of compounds, for example DL-methionine [1,2] and L-leucine [3]. The structural variations in the solid state of amino acids are a simple model for conformational dependent biochemical processes. In order to study the transitions for the group of the neutral aliphatic amino acids we have used differential scanning calorimetry (DSC), vibrational spectroscopy, and quantum mechanical calculations. In this paper the results of the DSC measurements of nine naturally occurring neutral aliphatic amino acids of and several structurally related compounds are reported. The 22 amino acids listed in Table 1 were examined in the temperature range 233–423 K.

EXPERIMENTAL

The thermal analysis was performed with a Perkin-Elmer DSC-2 calorimeter using steel pans. The heating rate was 10° min^{-1}. The heats of transition

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TABLE 1

Phase transitions of naturally-occurring neutral aliphatic amino acids and structurally related compounds

Compound	R [a]	D.l. [b]	T(K)	ΔH(kJ mol^{-1})	ΔS(kJ K^{-1} mol^{-1})	k_2/k_1
DL-α-Amino-*n*-butyric acid	CH_3-CH_2-	+	201 [6] 337 [7]			
L-α-Amino-*n*-butyric acid	CH_3-CH_2-	?	356	0.53	1.49	1.19
DL-Serine	$HO-CH_2-$	–				
L-Serine	$HO-CH_2-$	–				
DL-Cysteine [c]	$HS-CH_2-$	?	283 [9]	1.36 [9]	4.81	1.78
L-Cysteine	$HS-CH_2-$	+				
DL-Norvaline	$CH_3-(CH_2)_2-$	?	273	0.04	0.16	1.02
L-Norvaline	$CH_3-(CH_2)_2-$	?				
DL-Valine	$CH(CH_3)_2-$	+				
L-Valine	$CH(CH_3)_2-$	+				
DL-Threonine	$CH_3-CH(OH)-$	?				
L-Threonine	$CH_3-CH(OH)-$	–				
DL-Norleucine	$CH_3-(CH_2)_3-$	+	390	4.41	11.31	3.90
L-Norleucine	$CH_3-(CH_2)_3-$	+	389	0.11	0.28	1.03

DL-Leucine	$CH(CH_3)_2-CH_2-$	+				
L-Leucine	$CH(CH_3)_2-CH_2-$	+	352 [d]	0.20 [d]	0.57	1.07
L-Isoleucine	$C_2H_5-CH(CH_3)-$	+				
DL-Methionine	$CH_3-S-(CH_2)_2-$	+	[e]			
			326 [f]	0.82	2.51	1.35
			380			
L-Methionine	$CH_3-S-(CH_2)_2-$	+	307 [g]	1.98	6.45	2.17
			393	0.15	0.38	1.05
DL-Cystine	$-CH_2-S-S-CH_2-$	?				
L-Cystine	$-CH_2-S-S-CH_2-$	−				
meso-Cystine	$-CH_2-S-S-CH_2-$	?				

[a] The general formula of neutral aliphatic amino acids is $R-CH(NH_2)-COOH$ or $R[CH(NH_2)-COOH]_2$ for cystine.

[b] D.l. = Double layer. Most of the neutral aliphatic amino acids crystallize at least in one modification in double-layer structure: +, double layer structure; −, no double layer structure; ?, unknown structure.

[c] Data could not be reproduced.

[d] Bougeard [3]: 353 K, 0.18 kJ mol^{-1}.

[e] By vibrational spectroscopy and DSC-measurement the phase transition is restricted to the range between 98 and 233 K.

[f] Taniguchi et al. [2]: 326 K.

[g] Hutchins et al. [8]: 306 K.

were determined by cutting out and weighing the registered peaks. The temperature scale and the enthalpy were calibrated with indium as standard material. The accuracy of the measurement was $\Delta T = \pm 1$ K for the phase transition temperature and $\Delta H = \pm 5\%$ to $\pm 14\%$ for the corresponding enthalpy.

The amino acids of chromatographically homogeneous purity grade were obtained in polycristalline form from Fluka AG except DL-valine (E. Merck) and L-valine (Baker Chemikalien) and were not further purified. The compounds did not contain any crystal water (no mass-loss by heating for three days at 400 K).

CRYSTAL STRUCTURE OF NEUTRAL ALIPHATIC AMINO ACIDS

All crystals of neutral aliphatic amino acids studied by neutron diffraction occur as zwitterions [4]. The CO_2^-- and NH_3^+-groups are bound by hydrogen bonds; in this way most of the compounds build a double-layer structure. The alkyl side chains are nearly perpendicular to the double layers and neighbouring layers are held together by dispersions forces. The X-ray examination of phase transitions indicated three possible kinds of variation in the crystal structure:

(1) a conformational change in the alkyl chain [1,2];

(2) a rearrangement of the layers along the stacking direction (for example a translation of every second layer like in DL-methionine [1,2]);

(3) the appearance of a completely different hydrogen-bond network like in glycine [5].

Five of the 22 examined amino acids are known to undergo phase transitions in the solid state [1–3,6–10]. DL-α-Amino-*n*-butyric acid exhibits two transformations [6,7] and DL-methionine one at 326 K [1,2]. Both compounds have been studied by X-ray crystallography. The phase transition of L-methionine at $T = 307$ K was observed by heat capacity investigations [8]. L-Leucine [3] and DL-cysteine [9,10] show transitions which were examined by DSC-measurements and vibrational spectroscopy.

RESULTS

The measured DSC curves near the transition points are represented in Figs. 1–3. The observed phase transition enthalpies, temperatures and entropies are summarized in Table 1. This table is completed by structural data of amino acid phase transitions given in the literature [2,3,6–9]. The values of the transition entropy ΔS can be obtained from the measured transition enthalpy ΔH by

$$\Delta S = \Delta H / T$$

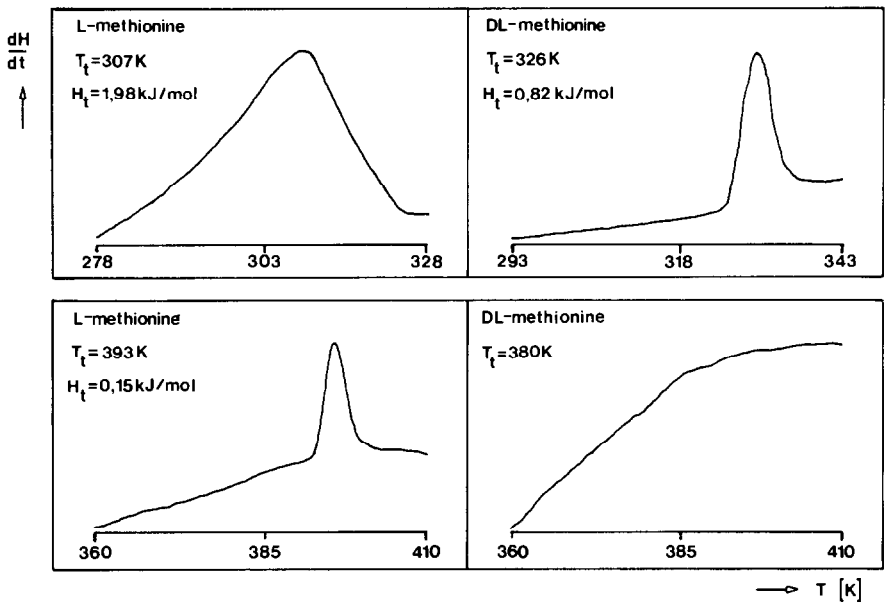

Fig. 1. DSC curves of methionine in the phase transition region.

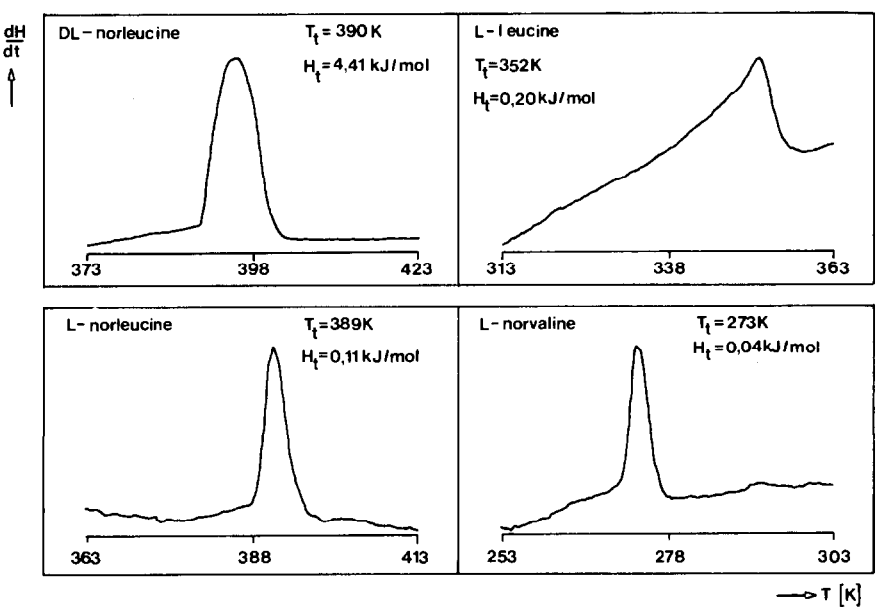

Fig. 2. DSC-curves of norleucine, leucine, and norvaline in the phase transition region.

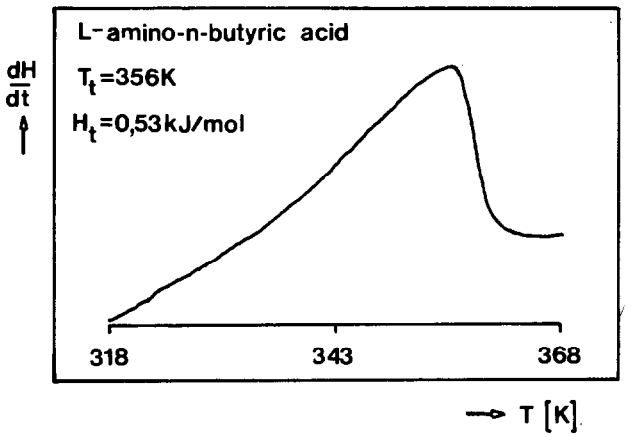

Fig. 3. DSC-curve of amino-butyric acid in the phase transition region.

Further, with the assumption that the transition is only of order–disorder type the occupation ratio k_2/k_1 can be obtained by the expression given by Westrum and McCullough [11]

$$k_2/k_1 = \exp(\Delta S/R)$$

where k_2/k_1 is the ratio of the number of states statistically occupied in a high and low temperature phase. ΔS and k_2/k_1 are listed in the two last columns of Table 1.

DISCUSSION

The observed phase transition enthalpies and temperatures agree with literature data considering the accuracy of the measurements [2,3,8]. Three phase transitions of aliphatic amino acids could not be observed by DSC:

(1) the transition to the low-temperature form of DL-α-amino-*n*-butyric acid at 201 K [6] is outside the measured temperature range (233–423 K);

(2) the rate of the transition (*A*- to *D*-form) of the same compound at 337 K is very slow [7];

(3) the absence of the phase transition of DL-cysteine at 283 K [9,10] could be explained by the fact that this compound easily undergoes an oxidation to the dimer cystine in the presence of light, traces of oxygen or metal ions [12]. Therefore, infrared investigations were carried out. The spectra show no variation before and after the DSC measurement. No cystine or decomposition products could be spectroscopically observed. Furthermore, the infrared spectra show a small variation compared to the infrared spectra published by Madec et al. [10]. At 1001 cm^{-1} in the present spectra a medium peak was found which is absent in the published spectra. The reason for this discrepancy is not known, up to now.

The third column of Table 1 states whether or not the listed amino acids crystallize in double layers at least in one crystal modification. Most of the compounds with double-layer structure show phase transitions. On the contrary, no transition is observed for amino acids which have no double-layer arrangement in any phase: DL-serine, L-serine, L-threonine, and L-cystine. Also, the structurally related amino acids, for which the crystal structure is unknown (DL-threonine, DL-cystine, and meso-cystine) show no phase transitions. Therefore, it can be assumed that DL-threonine builds a three-dimensional network of hydrogen bonds like L-threonine. For cystine it is impossible to build double layers because both ends of the side chains are connected by chemical S–S-bonds [13,14].

Also the two valine modifications L and DL, both with double-layer structure, show no phase transitions. This is possibly due to the length of the side chain: So far, all examined transitions of aliphatic amino acids show a change of the conformation around the β–γ-, γ–δ-bond. No torsion around the C^{α}–C^{β}-bond is observed. The side chains of DL-valine and L-valine are too short to show possible torsions.

Only L-methionine and DL-norleucine have $k_2/k_1 \geqslant 2$. Under the assumption that simple ratios like 2 : 1, 3 : 1, etc., occur, only these two transitions could be of the order–disorder type. Further, the vibrational spectra of L-methionine suggest the existence of a transition between 98 and 233 K.

CONCLUSION

In this report the DSC measurements of 22 crystalline aliphatic amino acids between $T = 233$ and 423 K were performed. Nine phase transitions could be observed in this temperature range. Only three of them were previously known. From observations made by X-ray analysis and vibrational spectroscopy there are 13 phase transitions of crystalline neutral aliphatic amino acids.

The investigation suggests that the phase transitions occur without any order–disorder effect and are caused by changing the side-chain conformation and by translations of double layers. Further investigations, especially by vibrational spectroscopy, are in progress in order to get a better insight in the mechanism of these phase transitions.

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