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Thermoanalytical and Calorimetric Studies on Polymorphic Behaviors of Crystalline Modifications in Aliphatic α-Amino Acids with Normal Alkyl Chains

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The B–A transition in DL-ABA occurs in the wide range of temperature near its decomposition temperature, and the transition pattern is similar to that of γ–α transition in glycine, whereas the pattern of α–γ transition in DL-NL is different from that in glycine, and the α–γ transition of DL-NL is of the first order. The enthalpy difference between modifications A and B of DL-ABA was nearly equal to the enthalpy of transition determined by DSC.

Much attention has been directed to polymorphic transition of aliphatic α-amino acids with normal alkyl chains, including glycine. Only the following compounds, however, have been studied. According to X-Ray diffraction and IR absorption studies, many of the aliphatic α-amino acids have crystalline modifications.[1–5] For example, glycine has 3 crystal modifications called α-, β-, and γ-forms, and the γ-form (trigonal) is transformed into the α-form (monoclinic) in the temperature range between 433 and 483 K. The enthalpy of transition is of the order of 1 kJ mol^{-1}. The transition is inferred to be due to the rearrangement of molecular layers bound with hydrogen bonds between the constituent molecules.[6–8] Iitaka and others found that the B-form of DL-2-aminobutanoic acid(DL-ABA) is transformed into the A-form at 468 K, and X-Ray analysis showed that the γ-carbon atom of the A-form DL-ABA (monoclinic) distributes among anti-position, gauche I, and gauche II with respect to nitrogen atom, whereas the γ-carbon atom in the B-form (tetragonal) is restricted to the anti-position.[6] As for polymorphic transitions of the B-form of DL-ABA and the α-form of DL-norleucine(DL-NL), it is considered that the enthalpy of transition includes contributions of rearrangement of molecular layers due to intermolecular interactions between constituent molecules and conformational change due to the rotation of carbon–carbon bonds in alkyl chains.

The thermodynamic study of phase equilibrium (or transition phenomenon) between crystal modifications seems to be useful together with the structural study of polymorphism, because the combination of structural analysis with thermodynamic study provides a significant information for polymorphism. In particular, the thermal analysis on solid-solid phase transitions between crystalline amino acids brings about suggestive information on transition phenomena of biological systems, especially of biomembranes. The objective of this work is to obtain information on polymorphic modifications of DL-ABA and DL-NL in comparison with the case of glycine by means of differential scanning calorimetry(DSC) and solution calorimetry.

Experimental

Materials. DL-2-Aminobutanoic acid(DL-ABA) and DL-norleucine(DL-NL) were purchased from Tokyo Kasei Co. and glycine from Takara Kohsan Co. All the samples used were the purest reagents commercially available: the purity of the compounds was guaranteed by each manufacturer to be 98%. The DL-NL was obtained as plate-like crystals by standing at 290 K for 24 h and the fibrous DL-ABA from a water-ethanol solution at 283 K for 48 h. By X-Ray diffraction and IR absorption, the DL-NL and DL-ABA samples were identified as α-form and B-form, respectively. The A-form DL-ABA was obtained as tabular crystals from aqueous solution by keeping at 283 K for 24 h, and the α-form glycine as tabular crystals by evaporating water. The A-form DL-ABA and α-form glycine were dried over silica gel for two months, and were dried again in vacuo for 6 h before use.

Apparatus and Procedure. Differential scanning calorimeter used in this experiment is Perkin Elmer DSC-II with Thermal Analysis Data Station(TADS). A few mg of sample was sealed in an aluminum pan, and the sample and the blank pans were set in each respective holder. The measurements of temperature and enthalpy were made in the

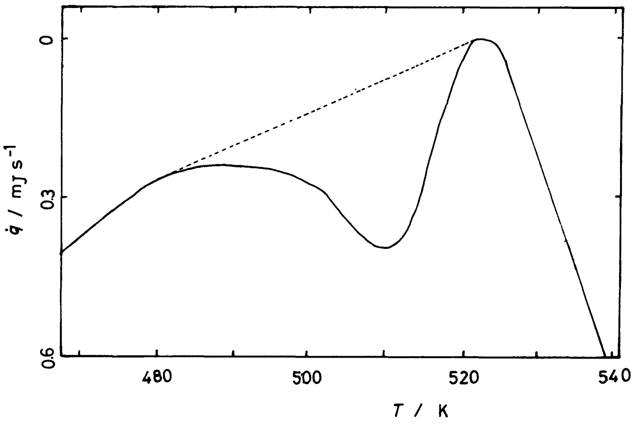

Fig. 1. DSC curve of the B-form of DL-ABA crystals heated at 5 K min^{-1} in the atmosphere of nitrogen. Sample weight: 2.64 mg.

Table 1. Thermodynamic Parameters for the Solid–Solid Transitions of Glycine and DL-2-Aminobutanoic Acid (DL-ABA)

Phase transition	Peak temperature T/K	Enthalpy change ΔH_t/kJ mol^{-1}	Entropy change ΔS_t/J mol^{-1} K^{-1}
Glycine	473(433—483)	0.92 ± 0.08[a]	1.95
$(\gamma\rightarrow\alpha)_{trs}$	478(459—491)	1.94 ± 0.06	4.06
DL-ABA			
$(B\rightarrow A)_{trs}$	506(480—520)	4.91 ± 0.29	9.70

a) Ref. 8.

range of heating rate between 0.63 and 10 K min^{-1}. The calorimeter was calibrated with indium, T_f=429.78 K and ΔH=28.5 J g^{-1}, supplied by Perkin Elmer Co. The melting point and enthalpy of fusion of indium obtained by extrapolating to null heating rate, was 429.6±0.1 K and 29.3±0.4 J g^{-1}, respectively. The apparatus for measuring enthalpy of solution was the same as described in the previous work.[9] The infrared spectra and X-Ray diffraction patterns were determined by model DR-31L of Japan Spectroscopic Co. and model JDX-8P of JEOL, respectively. Specific heat capacity measurements were carried out by model SH-3000M of Shinku-Riko Co. at heating rate of 1.5 K min^{-1}. The accuracy of the heat capacity apparatus was ±3%.[10]

Results and Discussion

DSC Measurements of DL-2-Aminobutanoic Acid(DL-ABA). The DSC result for DL-ABA is shown in Fig. 1. Phase transition from B- to A-form is observed in the temperature range between 480 and 520 K. The B-A transition of DL-ABA occurs near its decomposition temperature (577.15 K), and the transition pattern is similar to that of γ-α transition of glycine. The rate of the B-A transition of DL-ABA was slow similarly as the γ-α transition of glycine. Tsuboi et al. confirmed by means of IR spectra that the B-form was transformed into the A-form.[2] IR spetcrum of DL-ABA after transition was identical with that of A-form obtained by them. For comparison, the enthalpy of transition of glycine from γ-form to α-form was measured. The enthalpy value of transition of glycine was compared with that of DL-ABA at the same heating rate of 5 K min^{-1}. The enthalpy change of the B-A transition of DL-ABA was obtained by calculating the area between DSC curves at rise and fall (dotted line) of temperature. The results obtained are summarized in Table 1, where the enthalpy data are the average of 3 runs. Table 1 shows that the apparent entropy change of the B-A transition of DL-ABA is more than twice as that of the γ-α transition of glycine, where the entropy change was estimated by a calculation with the enthalpy change and peak temperature. Large entropy change in the B-A transition of DL-ABA seems to relate to the fact that the compound aquires the rotation around carbon-carbon bond in alkyl chain in solid state.[6] Furthermore, to confirm the value of enthalpy of transition obtained by DSC, the measurement of molar heat capacity of the B-form of DL-ABA was made. In Fig. 2, it appears that molar heat capacity, C_p, exhibits a steep peak and that enthalpy content, H, changes continuously. This feature is similar to that of specific heat capacity of $CuCl_2 \cdot 2H_2O$,[11] and seems as if C_p shows a discontinuity, that is a second-order phase transition. However, in view of the fact that the transition is accompanied by a volume change,[6] C_p and H at the transition temperature is considered to be continuous and discontinuous, respectively. Therefore, this feature makes us conclude that the transition is of the first order. As is shown in Fig. 2, the enthalpy of transition was estimated by extrapolation from both the lower and higher temperature regions to be approximately 5 kJ mol^{-1} at the transition temperature of 481 K.

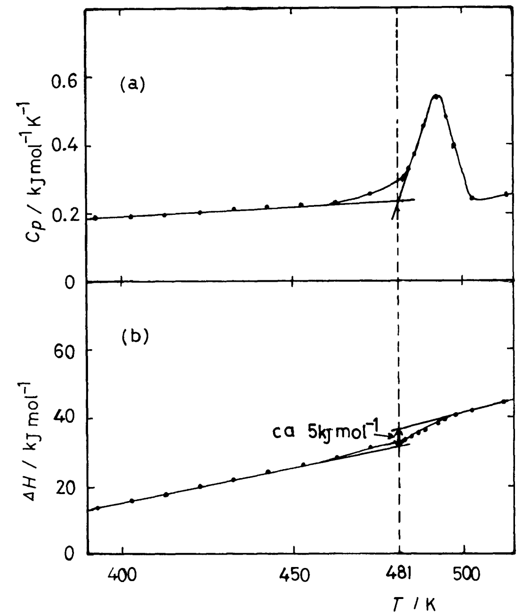

Fig. 2. Molar heat capacity (a) and enthalpy change (b) during the B-A transition in DL-ABA. Sample weight: 1.392 g. Heating rate: 1.5 K min^{-1}.

Solution Calorimetry of DL-2-Aminobutanoic Acid (DL-ABA). It is known that the A-form of DL-ABA is metastable at room temperature, whereas the B-form of DL-ABA is stable below the decomposition temperature (577.15 K). Since it is expected that the difference in the enthalpy of solution at room temperature between A and B is comparable to the enthalpy of transition of B- to A-form of DL-ABA obtained above, the

Table 2. Calorimetrically Determined Enthalpy Values of Aqueous Solutions of Glycine and DL-ABA at 298.15 K

Substance	Molality range $m/\mathrm{mol\,kg^{-1}}$	Enthalpy of solution $\Delta H_s/\mathrm{kJ\,mol^{-1}}$	Author
α-form of	0.3	15.0±0.2	K. Sasaki[8]
Glycine	0.01	11.6±0.2	This work
γ-form of	0.3	15.8[a]	K. Sasaki[8]
Glycine	0.01	13.4±0.4	This work
A-form of	3×10^{-3}	1.56±0.01	Abraham et al.[12]
DL-ABA	5×10^{-3}	1.69±0.03	This work
B-form of	3×10^{-3}	6.92±0.08	Abraham et al.[12]
DL-ABA	5×10^{-3}	6.66±0.05	This work

a) One determination only.

Table 3. Difference in the Enthalpy of Solution between Crystalline Modifications at 298.15 K

Substance	Enthalpy difference $\Delta\Delta H_s/\mathrm{kJ\,mol^{-1}}$	Author
Glycine	0.8	K. Sasaki
	1.8±0.6	This work
DL-ABA	5.36±0.09	Abraham et al.
	4.97±0.08	This work

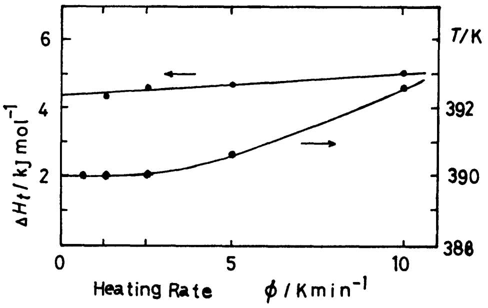

Fig. 3. Heating rate dependence of the enthalpy of transition, and of the peak temperature in the α-γ transition of DL-norleucine.

enthalpy of solution of crystal modifications of DL-ABA was measured at 298.15 K. The results obtained are given in Tables 2 and 3 together with data of preceding authors. The enthalpy of solution of glycine was measured for reference. The enthalpy of solution of both the crystal modifications in water at 298.15 K was determined by an isoperibol calorimeter. As was shown in a previous work, the systematic errror of the calorimeter was 1.4%, and the accuracy was 0.34%.[9] The enthalpy was compared between the final concentrations of glycine and DL-ABA, i.e., 1×10^{-2} and 5×10^{-3} mol kg^{-1}, respectively. The average value of enthalpy of solution for 5 determinations was used. The enthalpy data obtained agree fairly well with those of Abraham et al.[12] The difference in the enthalpy of solution between α- and γ-forms of glycine is more than twice as that obtained by Sasaki.[8] This may be due to the difference of apparatus used and purity of α-glycine used for the solution calorimetry. Sasaki pointed out that the enthalpy difference in two forms of glycine can be ascribed to the difference in intermolecular hydrogen bonds caused by rearrangement of molecular layers.[8] Assuming that the contribution of the difference in intermolecular hydrogen bonds of two forms of DL-ABA is nearly equal to that of glycine polymorphs, the difference of intermolecular hydrogen bonds is about 2 kJ mol^{-1}. The data in Table 3 suggest that the contribution due to intramolecular interactions, such as rotation around carbon-carbon bonds in alkyl chain, is predominant in the case of DL-ABA. From Tables 1 and 3, the difference in enthalpy of solution between two forms of DL-ABA appears to be nearly equal to the enthalpy of transition determined by DSC. This fact may be explained as follows. The enthalpy difference between two forms, $\Delta\Delta H_s$, is expressed as

$$\Delta\Delta H_s = \Delta H_t - \int_{T_s}^{T_t} \Delta C_p \mathrm{d}T, \tag{1}$$

where ΔC_p is the difference between molar heat capacity values at transition temparature, T_t, and at temperature of solution calorimetry, T_s. Experimental results show that $\Delta\Delta H_s$ is nearly equal to ΔH_t. The ΔC_p is negligible in the case of DL-ABA, but ΔC_p in glycine is not negligible for small quantity of enthalpy.

DSC Measurements of DL-Norleucine (DL-NL). The previous work[13] reported that a solid-solid phase transformation of crystalline DL-NL was displayed by means of DSC and X-Ray diffraction. As is shown in Fig. 3, the values of peak temperature depend on the heating rate, but the enthalpy of transition is nearly constant. The transition temperature and enthalpy of transition are estimated to be 390 K and 4.4 kJ mol^{-1}, respectively, by extrapolation to null heating rate.

On the other hand, Grunenberg et al. observed the transitions for the group of crystalline neutral apliphatic α-amino acids by means of DSC, infrared absorption spectra, and Raman spectra;[14,15] the transition of DL-NL occurs at 390 K and the enthalpy of transition is 4.41 kJ mol^{-1} at a heating rate of 10 K min^{-1}. Although the form of DL-NL was not identified as α-form by Grunenberg et al., our values are in good agreement with those by them. Therefore, the

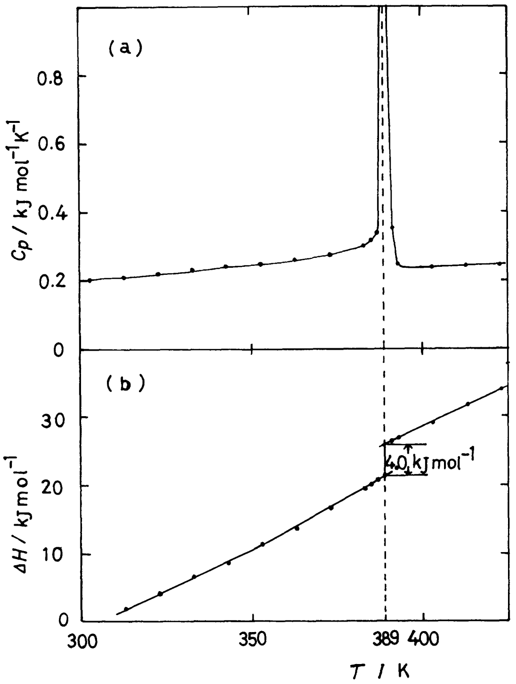

Fig. 4. Molar heat capacity (a) and enthalpy change (b) during the α-γ transition in DL-norleucine. Sample weight: 2.841 g. Heating rate: 1.5 K min^{-1}.

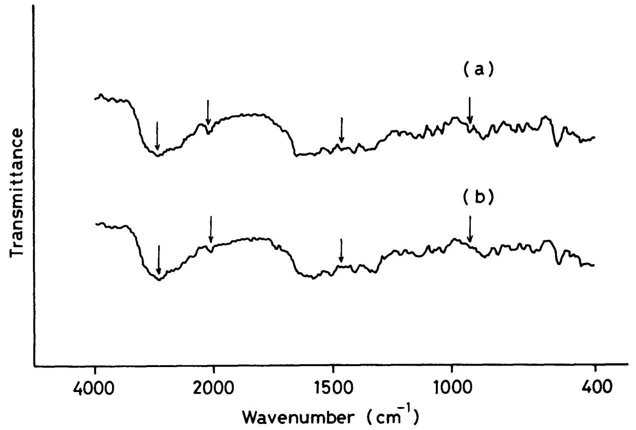

Fig. 5. Infrared absorption spectra of DL-norleucine at 353 K (a) and at 443 K (b).

α-form DL-NL seems to be transformed into a new form called γ-form at 390 K.

In order to know the nature of transition of DL-NL, the specific heat capacity was measured. The sample weight was 2.841 g. As is shown in Fig. 4, the α-γ transition of DL-NL was found to be of the first order.[16] Figure 4 indicates that the transition temperature and the enthalpy of transition correspond to those by DSC measurements. The transition of DL-NL is reversible, whereas the γ-α transition of glycine and the B-A transition of DL-ABA are irreversible. Therefore, the free energy curves should intersect at the α-γ transition temperature, T_t. The entropy change in the α-γ transition of DL-NL by DSC was estimated to be 11 J mol^{-1} K^{-1}. The transition will be characterized by a large structural change similarly to the B-A transition in DL-ABA (9.70 J mol^{-1} K^{-1}, see Table 1).

Infrared Absorption Spectra and X-Ray Diffraction of DL-NL. Other physical methods such as X-Ray diffraction and IR absorption spectra were used for analysing structure change above and below the transition temperature of DL-NL.[13] The X-Ray pattern obtained was identical with that of the α-form crystals reported by Dawson and Mathieson (J.C.P.D.S. Powder Diffraction File No. 7-717).[17] Also IR spectrum of the α-form DL-NL at room temperature was identical with that of known structure.[18] The existence of polymorphism of DL-NL was suggested by Mathieson, who deduced it to be the β-form or the superlattice from the dimorphs of DL-methionine.[19–21] X-Ray diffraction result was different from the spacings either of β-form or of the superlattice. Also IR spectra of DL-NL crystals measured above and below the transition temperature are given in Fig. 5. The spectral pattern at 443 K(b) is different from that at 353 K(a). In the wavenumber range of 2000—3000 cm^{-1}, the band of the α-form (a) at 2100 and 2950 cm^{-1} were assigned to be of NH_3^+ stretching,[18] and these bands shifted to shorter wavenumbers by about 20 and 50 cm^{-1} at 443 K(b). Accordingly, it is considered that intermolecular hydrogen bond distance between NH_3^+ and COO^- groups in the γ-form is shorter than that of the α-form. Also the bands at 1470 and 925 cm^{-1} in the α-form are assigned to be of CH_3 asymmetric deformation and CH_3 rocking plus CC stretching, respectively.[18,22] The changes of the bands were observed at 443 K(b). The IR spectrum change obtained is similar to that of L-leucine reported by Bougeard.[15] The spectral change suggests that the α-γ transition in DL-NL is caused by the change of intermolecular interactions between layers accompanied by the conformational change of alkyl chains.

Application of the Enthalpy of Solution to the Analysis of Mixtures of A- and B-Forms of DL-ABA. The enthalpy of solution of DL-ABA in water has been measured calorimetrically by some investigators, but the values obtained were different to each others.[23–25] The different solution enthalpy data can be ascribed to samples which are mixtures of A- and B-forms. Plate-like A-form crystals are easily obtained by recrystallization from water or water-ethanol mixture, but it is difficult to obtain pure B-form uncontaminated with the A-form as demonstrated by Abraham et al.[12] A sample of DL-ABA was regarded as pure B-form, because the IR spectrum of fibrous DL-ABA was identical with that given by Tsuboi et al.[2]

If the sample used is a mixture of A- and B-forms, the molar enthalpy of solution, $\Delta H^{\mathrm{m}}_{\mathrm{s}}$, is expressed by

$$\Delta H^{\mathrm{m}}_{\mathrm{s}} = (1-x)\Delta H^{\mathrm{A}}_{\mathrm{s}} + x\Delta H^{\mathrm{B}}_{\mathrm{s}}, \tag{2}$$

where x is mole fraction of B-form in DL-ABA, and $\Delta H^{\mathrm{A}}_{\mathrm{s}}$ and $\Delta H^{\mathrm{B}}_{\mathrm{s}}$ is molar enthalpy of pure A- and B-

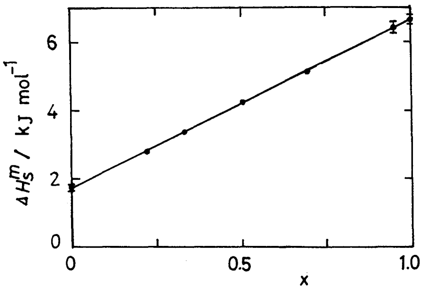

Fig. 6. Plots of the enthalpy of solution of mixture, ΔH_s^m, against mole fraction of the B-form, x, in DL-ABA at 298.15 K. It shows that Eq. 2 is found to hold as ΔH_s^m variation of arbitrary mixing ratios on mole fraction gives straight line.

form, respectively.

To confirm whether Eq. 2 is hold, the solution calorimetry of mixing of A-form with B-form was performed at 298.15 K. Figure 6 shows linear relationship between ΔH_s^m and ΔH_s^A (or ΔH_s^B). Accordingly, we can estimate the mole fraction of a component in the mixture from the enthalpy data obtained by mixtures of known mole fractions. ΔH_s^A and ΔH_s^B values are given in Table 2.

For example, the crystallization from ethanol-water mixture at 290 K for 24 h gave ΔH_s^m of 6.43±0.14 kJ mol^{-1} at 298.15 K as the average of 3 determinations; Eq. 2 gave the result that the mixture consists of 95% B-form and 5% A-form.

Conclusion

The polymorphic transitions of DL-2-aminobutanoic acid(DL-ABA) and DL-norleucine(DL-NL) were studied by differential scanning calorimetry(DSC). The experimental results show that the B-A transition in DL-ABA occurs in the wide range of temperature near its decomposition temperature, and the transition pattern is similar to that of γ-α transition in glycine, whereas the pattern of α-γ transition in DL-NL is different from that in glycine. The DL-NL transition is of the first order. The solution calorimetry of crystalline modifications of DL-ABA was performed by an isoperibol calorimeter at 298.15 K to reveal that the enthalpy difference is nearly equal to the enthalpy of transition by DSC. The α-γ transition in DL-norleucine is accompanied with change of intermolecular interactions such as hydrogen bond and van der Waals force between layers caused by the conformational change of alkyl chains. The correlation between the number of carbon atoms in alkyl chains of amino acids and the apparent entropy of transition is shown in Fig. 7.

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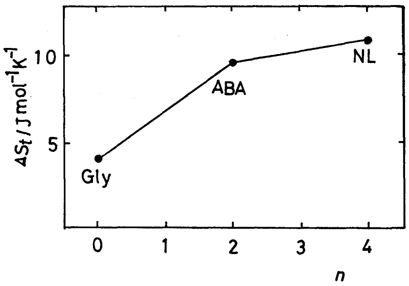

Fig. 7. Correlation between number of carbon atoms, n, in the alkyl chains of amino acids and apparent entropy of transition.

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