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Phase Transition of *N*-Acyl Amino Acids with Different Acyl Group

Shigeyoshi MIYAGISHI,* Seiichi MATSUMURA, Tsuyoshi ASAKAWA, and Morie NISHIDA
Department of Industrial Chemistry, Faculty of Technology, Kanazawa University, Kanazawa 920
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The solid-liquid phase transitions of *N*-acyl amino acids with different acyl groups were investigated using a differential scanning calorimeter and an infrared spectrophotometer. The enthalpy and entropy changes of the transition increased by 3—5 kJ mol^{-1} and 8—10 J $K^{-1}mol^{-1}$, respectively, for an additional methylene group in *N*-acylglycine, and 7 kJ mol^{-1} and 18 J $K^{-1}mol^{-1}$, respectively, in the *N*-acyl-L-alanine series. The same thermodynamic quantities of *N*-acylvalines were only slightly dependend on the acyl chain length, while they varied irregularly for the *N*-acylleucine series. Both the enthalpy and entropy were smaller for the L-form than for its racemic isomer in the *N*-acylleucines (C_{12} and above) and *N*-acylvalines. The differences of the thermodynamic quantities between the L- and DL-forms seems to result mainly from the difference of the molecular packing in the solid state.

Chirality of molecules is one of the most important factors in determining the molecular packing in the solid state. For compounds with the long hydrocarbon chain, it is known that both the packing of the chains[1] and the degree of hydrogenbonding[1,2] depend on the chirality. Tachibana and his coworkers[1,3-5] found that 12-hydroxyoctadecanoic acid exhibited a chirality effect in a monolayer state, as well as in the solid state. Such chiral aggregation phenomena have often been found in monolayers,[6-10] ion aggregations,[11] micelles,[12,13] and liquid crystals.[14-16] It has also been reported that the liquid state of racemic 2-octanol differs considerably from that of the enantiomers in its physical properties.[17] Our previous paper[18] concerning the phase transition of *N*-acyl amino acids indicated that the chirality effect depended on the amino acid residue.

In the present work, the dependence of the chirality effect on an acyl group was examined with a differential scanning calorimeter and an infrared spectrophotometer.

Experimental

N-Acyl amino acids were prepared and purified according to a procedure described previously.[13,18,19] *N*-acylglycines were recrystallized from a mixture of ethanol and petroleum ether, and the other *N*-acyl amino acids were recrystallized from a mixed solvent of diethylether and petroleum ether. A differential scanning calorimeter (Daini Seikosha DSC,

Table 1. Properties of *N*-Acyl Amino Acids

	Transition Point			Melting Point		
	T_t/K	ΔH_t/kJ mol^{-1}	ΔS_t/J $mol^{-1}K^{-1}$	T_m/K	ΔH_f/kJ mol^{-1}	ΔS_f/J $mol^{-1}K^{-1}$
N-Dec-Gly				387.6	42.2	109
N-Dodec-Gly[a]				393.1	48.4	123
N-Tetradec-Gly	379.6	6.8	17.9	396.6	47.4	120
N-Hexadec-Gly	384.6	4.5	11.7			
	366.1	5.6	15.2	393.1	56.5	144
N-Dodec-L-Ala[a]				356.1	37.6	106
N-Tetradec-L-Ala				367.1	52.3	143
N-Hexadec-L-Ala				374.1	65.3	174
N-Dec-L-Val	378.1	21.3	56.4	380.6	15.4	40.4
N-Dodec-L-Val[a]				380.1	33.1	87.1
N-Tetradec-L-Val	334.6	14.9	14.2	365.1	20.6	56.4
N-Hexadec-L-Val	349.1	29.1	83.4	366.6	54.8	70.0
N-Dec-DL-Val				358.1	63.1	176
N-Dodec-DL-Val[a]				364.6	64.4	177
N-Tetradec-DL-Val				370.1	68.1	184
N-Hexadec-DL-Val				375.1	80.5	214
N-Oct-L-Leu	357.1	7.6	21.4	398.1	29.3	73.7
N-Dec-L-Leu	343.1	1.2	3.4	383.1	27.5	68.8
N-Dodec-L-Leu				383.1	33.5	87.4
N-Tetradec-L-Leu				377.6	32.4	85.8
N-Hexadec-L-Leu				367.1	46.1	126
N-Oct-DL-Leu	353.6	6.8	19.3	367.1	27.2	74.2
N-Dec-DL-Leu				357.1	28.9	81.0
N-Dodec-DL-Leu	341.1	28.9	84.8	356.6	31.0	86.9
N-Tetradec-DL-Leu	320.1	1.8	5.5	349.6	54.8	157
N-Hexadec-DL-Leu	333.1	4.3	12.8	355.1	60.6	171

a) From Ref. 18.

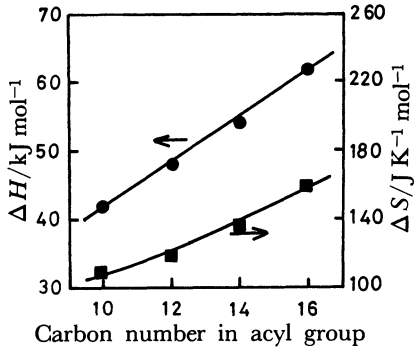

Fig. 1. Enthalpies and entropies of fusion of *N*-acylglycines.

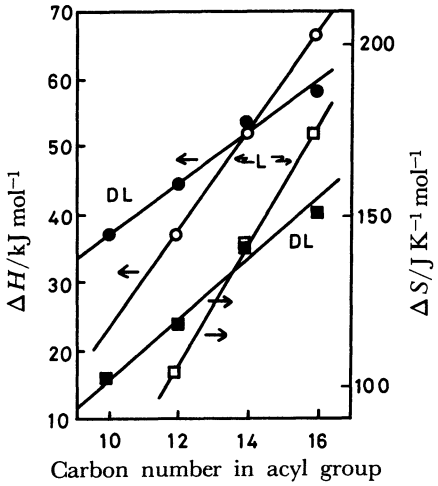

Fig. 2. Enthalpies and entropies of fusion of *N*-acylalanines.
The data of the racemic derivatives were cited from Ref. 18.

model SSC/560S) was used for measurements of the transition temperatures and their enthalpies under the same conditions as described previously.[18] Infrared(IR) spectra were recorded on a JASCO A202 spectrophotometer.

Results and Discussion

Thermal Analysis. The phase-transition temperatures, enthalpies, and entropies of the *N*-acyl amino acids are given in Table 1. The table shows that several *N*-acyl amino acids undergo a transition below the melting point. No distinct correlation existed between the thermodynamic quantities of the transition and the structure of the compound. If this transition is considered as a partial melting, the total enthalpy corresponding to a change from the solid to the liquid state should be the sum of the enthalpy of fusion and the transition enthalpy. Such summation should be true also for the entropy. The total enthalpy and entropy are plotted in Figs.1—4.

The total enthalpy(ΔH) and total entropy(ΔS) increased with the length of an acyl group in the *N*-acylglycine and *N*-acyl-L-alanine series. The increment

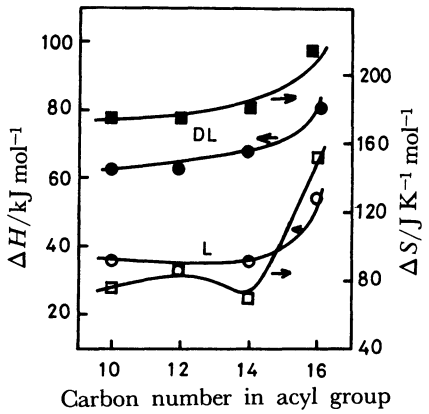

Fig. 3. Enthalpies and entropies of fusion of *N*-acylvalines.

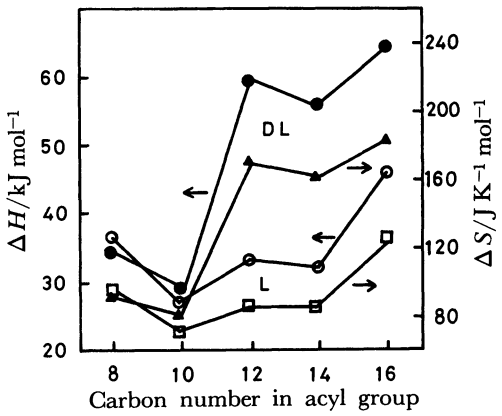

Fig. 4. Enthalpies and entropies of fusion of *N*-acylleucines.

per methylene group in ΔH and ΔS was 3—5 kJ mol^{-1} and 8—10 J K^{-1} mol^{-1}, respectively for the glycine derivatives. These figures are compatible with the corresponding values (4 kJ mol^{-1} and 9 J K^{-1} mol^{-1}) for normal paraffins,[20] monoglycerides,[21] and the racemic *N*-acylalanines.[18] The acyl groups in the monoglycerides and the racemic *N*-acylalanines were concluded to behave in the same manner as a normal alkane when they melted. Therefore, the acyl groups of the *N*-acylglycines seem to behave in the same manner as normal paraffins in regard to the phase transition.

For the L-alanine series, ΔH and ΔS increased by 7 kJ mol^{-1} and 18 J K^{-1} mol^{-1} respectively for each additional methylene group, as seen in Fig. 2. These values were abnormally large as compared with those of the racemic isomer.[18] This result implies a possibility for the acyl groups of the L-isomers to interact attractively with each other in the solid state or to do so repulsively in the liquid state.

In the *N*-acylvaline series, ΔH and ΔS increased slightly with the chain length of the acyl group (Fig. 3). The magnitude of their increments were small suggesting that the methylene chains were fusible or melted in the solid state. The change occuring in the amino acid part of the molecule may contribute to most

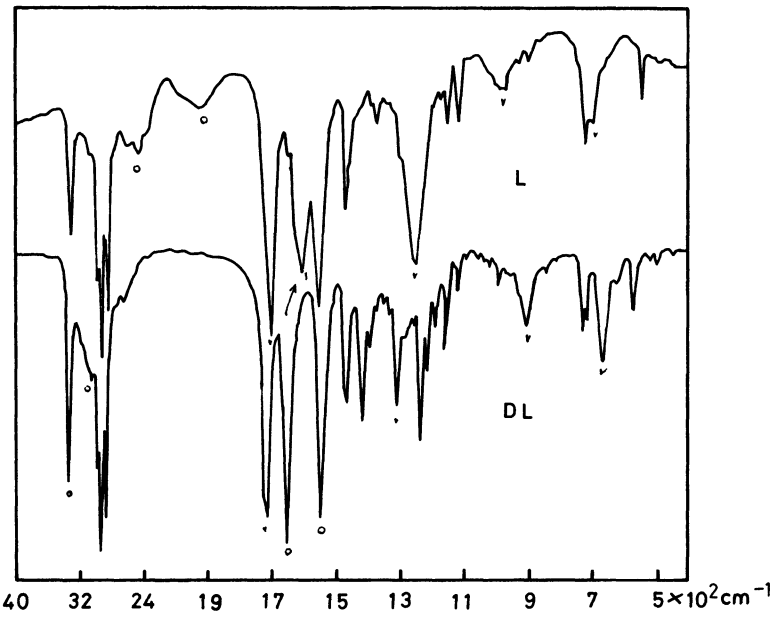

Fig. 5. IR spectra of *N*-tetradecanoyl-L-valine and -DL-valine.

of ΔH and ΔS, when the *N*-acylvalines undergo a transition from solid to liquid. The relation of the thermodynamic quantities with the acyl group was more complicated in the *N*-acylleucines. The enthalpy and the entropy varied in a zigzag form with the length of the acyl group (Fig. 4). Therefore we must consider that the acyl group and the amino acid part in the molecule interact strongly with each other when the phase transition occurs and the influence becomes larger as the molecular structure becomes more complicated.

Next we shall consider the chirality effect. In the alanine series, ΔH and ΔS increased linearly with the chain length of the acyl group, however, a difference was observed in the slope between the chiral and racemic series. For the compounds with the acyl chain shorter than a tetradecanoyl group, the racemic compounds gave larger ΔH and ΔS than the corresponding L-isomers, while *N*-hexadecanoyl-L-alanine had large values compared with its racemic isomer (Fig. 2). The differences between the values of the L- and DL-forms were smaller in the alanine series than in the valine and leucine series (as seen in Figs. 2—4).

Every racemic *N*-acylvalines gave larger ΔH and ΔS than the optically active isomers (as be seen in Fig. 3). In the *N*-acylleucine series, octanoyl and decanoyl derivatives respectively gave nearly identical values for both the chiral and racemic isomers, while the racemic derivatives with a longer acyl chain had large ΔH and ΔS compared with the chiral isomer. The experimental results indicate that if the liquid state is taken as the standard, the racemic form is thermally more stable and more ordered in the solid state than the chiral form, in the case of the *N*-acylvalines and the *N*-acylleucines (except for C_8 and C_{10}). Therefore the chirality effect depends not only on the structure of the amino acid residue but also on the chain length of the acyl group.

The specific heat of fatty acid (C_6—C_{25}) is 2.13—2.42 $J\,g^{-1}\,K^{-1}$ in the liquid state and 1.88—2.09 $J\,g^{-1}\,K^{-1}$ in the solid state,[22a] although the values of the specific heat of the *N*-acyl amino acids are not known. If the values for the *N*-acyl amino acids can be assumed to be similar to those of the fatty acids, the difference of the specific heat between the liquid and solid states is about 0.3 $J\,g^{-1}\,K^{-1}$. For the *N*-decanoyl valines (the melting point is 380.5 and 355 K for the L- and DL-forms), we get the value of 2 $kJ\,mol^{-1}$ (=0.3×(380—355)×271.4) as the enthalpy change due to the temperature difference. This value is negligibly small compared with the difference found in Fig. 3. This fact reveals that the differences between the thermodynamic quantities of the L- and DL-forms have some other origin.

IR Spectra. Information about the solid state is available from the measurement of its IR spectrum, because the spectrum is known to depend on its crystal structure. The IR spectra of the *N*-acyl amino acids were measured in KBr pellets in order to examine a difference of the solid state between the L- and DL-forms. Both the optically active and racemic *N*-acylalanines gave almost identical absorption characteristics except for the region from 600 to 700 cm^{-1}. The band at 690 cm^{-1} is ascribed to O-C=O angle deformation and the band at 640 cm^{-1} may be the C=O bending mode of the CONH group.[23] These two bands were replaced by a single band at 650 cm^{-1} in the racemic form.

Figure 5 shows that there is a significant difference between the spectra of the *N*-acyl-L-valine and -DL-valine. The strong bands at 1710, 1310, 900, and 660 cm^{-1} in the racemic form are associated with a carboxyl group.[24] The C=O stretching band at

1710 cm^{-1} and the C–OH stretching band at 1310 cm^{-1} in the racemic form shifted to 1700 and 1250 cm^{-1}, respectively, in the chiral form. The OH bending band at 900 cm^{-1} and the O–C=O angle deformation band at 680 cm^{-1} shifted to 980 and 695 cm^{-1}, respectively. On the other hand, the bands at 3310, 3100, 1650, and 1550 cm^{-1} in the DL-form are ascribed to amide A, B, I, and II bands respectively.[25] The amide A, B, and II bands are mainly associated with the N–H vibration, while the amide I band is largely a C=O stretching vibration. The amide I band at 1650 cm^{-1} in the DL-form shifted to 1600 cm^{-1} in the L-form, but the amide A, B, and II bands scarcely changed. The broad band near 3100 cm^{-1} in the racemic form was ascribed to hydrogenbonded OH vibration. The corresponding band in the L-form overlapped with the methyl and methylene stretching bands. In addition, two broad bands were observed at 1900 and 2500 cm^{-1} in the L-form.

It is well known that the hydrogenbonded OH band shifts to a lower frequency when the hydroxyl groups form multiple hydrogenbonding.[25] Furthermore the amide A and I bands shift to the low frequency direction and the amide II band to a higher frequency side when the strength of the hydrogenbonds increase owing to intermolecular association.[25] Chen and Parthasarathy[26] found from X-ray studies that short intermolecular hydrogenbonds between the carboxyl OH group and the *N*-acyl oxygen atom are an important feature for *N*-acyl amino acids and link the molecules in a head-to-tail fashion in an infinite chain. These facts suggest the following two possibilities. The small shift of the amide A, B, and II bands suggests that there is little difference in the contribution of the NH group to the hydrogenbonding between the L- and DL-forms. Some of the differences between the L- and DL-forms may result from a difference in the hydrogenbonding between the carboxyl OH group and the oxygen atom of the *N*-acyl group. However, the difference between the IR spectra seen in Fig. 5 is too large to be due to only the difference in the hydrogenbonding force. It is well known that the frequency of the amide I bands depends on the molecular conformation.[23,27,28] The authors reported in the previous paper[18] that the DL-derivatives of the *N*-acyl amino acids form racemic compounds in the solid state. Usually the IR spetrum and the crystal structure of the racemic compound differ significantly from those of the racemic mixture and the optically active isomers. We should also take into account the difference in the molecular packing between the L- and DL-forms. The optically active *N*-acyl amino acids gave different X-ray diffraction patterns from those of the racemic isomers.[29,30] The fatty acids usaully have an anti-parallel packing of the molecules in their solid states.

Therefore we assume two possibilities about the molecular packing in the L-form. The first case is the formation of a dimer or a molecular packing relatively similar to it. This dimer may be formed due to hydrogen bonding between the carboxyl OH group in one molecule and the *N*-acyl oxygen atom in the other molecule, and these molecules may be linked in an anti-parallel fashion to each other. In addition, such molecular packing can make the skeletal plane of the hydrocarbon chain parallel with its neighbors and enables us to explain a single peak at 720 cm^{-1} (see later). The second possibility is the formation of a similar bilayer structure as that found in the long chain fatty acids except for the hydrogen bonding. The hydrogenbonds in the latter case are formed between the carboxyl groups, but those in the *N*-acyl-L-valine may be formed between the carboxyl OH group and the *N*-acyl oxygen atom. In addition the bilayer structure may be linked to each other by the hydrogenbonds. In the racemic form the molecule has a different chirality from its neighbors. The valine residue group in the *N*-acylvaline may take up a trans position with respect to its NH group and thereby may prevent strong interaction between its *N*-acyl oxygen atom and the carboxyl group in its neighboring molecule compared with that in the L-from.

The IR spectrum of *N*-hexadecanoyl-L-valine was different from those of the other chiral *N*-acylvalines. Its spectrum was similar to those of the racemic derivatives in the region above 1500 cm^{-1} and was different in the regions of the methylene rocking and wagging bands. Furthermore the bands associated with the carboxyl group and the amide group had the same frequency and shape as those of the racemic isomer. *N*-Hexadecanoyl-L-valine seems to be different from the other *N*-acyl-L-valines in the solid state and to give larger thermodynamic quantities than the other (see Fig. 3). The spectra of the racemic *N*-acylvalines were almost the same as each other. This probably suggests that the racemic derivatives have closely similar crystal forms.

In the racemic *N*-acylvaline series, the methylene rocking vibration gives rise to split components at 715 and 725 cm^{-1}. In the L-forms a single rocking band which overlapped the carboxyl angle deformation band, was observed (Fig. 5). The *N*-acylglycines and *N*-acylleucines also exhibited a splitting of the methylene rocking band. On the other hand, the alanine derivatives gave a sharp single band at 720 cm^{-1} (Fig. 6). Such splitting of the methylene rocking band has been found in fatty acids,[24] normal paraffins,[31,32] 12-hydroxyoctadecanoic acid,[1] polyethylene,[33] and lithium *n*-hexadecanoate,[34] and is related to a hydrocarbon chain packing in the crystal. In the powder X-ray diffraction pattern of *N*-dodecanoyl-DL-valine,[30] the peaks at 4.11 and 3.72 A are in good agreement with those in the B- and C-forms of octadecanoic acid and the C-form dodecanoic acid, in which the methylene chain skeletal plane is nearly perpendicular to its neighbors in the orthorhombic subcell.[35]

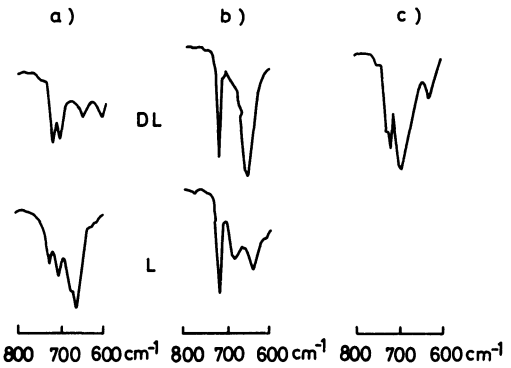

Fig. 6. Comparison of IR spectrum between L- and DL-isomers.
a) *N*-Tetradecanoylleucines, b) *N*-Tetradecanoylalanines, and c) *N*-Tetradecanoylglycine.

This agreement supports the nearly perpendicular packing of the methylene chains in *N*-dodecanoyl-DL-valine. Therefore the splitting in the methylene rocking band can be explained by such packing of the methylene chains. In its L-form, the peak corresponding to the hexagonal subcell packing of the methylene chains was not detected in the powder X-ray diffraction pattern.[30] Thus the parallel packing of the skeletal plane of the methylene chain may give the single peak at 720 cm^{-1}.

N-Octanoyl- and *N*-decanoyl-DL-leucines gave the same spectrum as those of the *N*-acyl-L-leucines. The spectra of the other racemic *N*-acylleucines differed from those of the chiral isomers. In the region above 1500 cm^{-1}, the consideration of the comparison of the L- and DL-forms in the valine derivatives was also applicable to the leucine series. In other regions, a band at 660 cm^{-1} in the L-form was replaced by two bands at 650 and 610 cm^{-1} in the DL-form, and the OH bending band at 920 and 970 cm^{-1} were replaced by a single band at 940 cm^{-1}.

The above results indicate that the *N*-acyl amino acids with large difference between the L- and DL-forms in the thermodynamic quantities also exhibit big differences in their IR spectra. The differences found in the IR spectra reflects that of the solid state, because the measurement of the spectra was done at room temperature. Analogy between the results of the thermal analysis and the spectral data indicates that the differences of the thermodynamic quantities between the L- and DL-forms result mainly from the difference of the molecular packing in the solid state.

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