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ORIGINAL CONTRIBUTION

Akio Ohta
Satoru Nakashima
Hiroki Matsuyanagi
Tsuyoshi Asakawa
Shigeyoshi Miyagishi

Krafft temperature and enthalpy of solution of *N*-acyl amino acid surfactants and their racemic modifications: effect of the counter ion

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A. Ohta (✉) · S. Nakashima
H. Matsuyanagi · T. Asakawa
S. Miyagishi
Department of Chemistry and Chemical Engineering, Faculty of Engineering, Kanazawa University, 2-40-20 Kodatsuno, 920-8667 Kanazawa, Ishikawa, Japan
E-mail: akio-o@t.kanazawa-u.ac.jp
Tel.: +81-76-234-4766
Fax: +81-76-234-4800

Abstract The Krafft temperatures and enthalpies of solution of *N*-hexadecanoyl alaninate and valinate, and *N*-tetradecanoyl phenylalaninate were obtained from differential scanning calorimetry. The Krafft temperature of *N*-acyl amino acid surfactant increased with decreasing size of the counter ion, with some exceptions. The enthalpy of solution was endothermic and increased with decreasing size of the counter ion except for the cases of lithium salt. The results showed that the L-L interaction in the solid state of *N*-hexadecanoyl amino acid surfactant salt was superior to the D-L interaction for both the alanine and valine systems when the counter ion size increased. However, the D-L interaction was still advantageous for the phenylalanine system with Cs^+ as a counter ion. Both Fourier transform infrared spectroscopy studies and theoretical calculations suggested that the difference in magnitudes of the interactions between peptide and counter ion was a dominant factor for the chiral effect.

Keywords *N*-Acyl amino acid surfactant · Krafft temperature Enthalpy of solution · Counter ion Chiral effect

Introduction

N-Acyl amino acid surfactants are useful from both industrial and domestic viewpoints because of their biodegradability and low toxicity [1, 2]. However, concerns with their chiral discrimination has been growing since it has been reported that some amphiphiles containing peptide groups such as *N*-acyl amino acid were self-assembled to micrometer- and nanometer-scale structures [3, 4, 5, 6, 7]. It has been known that the chiral discrimination of *N*-acyl amino acid surfactants occurs remarkably in concentrated molecular organizations like solids [8, 9, 10], liquid crystal [11, 12], and condensed interfacial film [13, 14, 15, 16, 17]. In an earlier study [10], we showed by measurements of Krafft temperature (KT) and the enthalpy of solution of sodium *N*-hexadecanoyl amino acid surfactant salt that, in the solid state, the heterochiral (D-L) interaction was superior to the homochiral one for both alanine (Ala)and phenylalanine (Phe) systems, while the homochiral (L-L or D-D) interaction was advantageous for both the valine (Val) and leucine systems. Further, we concluded that both peptide-peptide hydrogen bonding and steric hindrance of the amino acid residue govern the chiral effect. There are some studies on crystal structures of *N*-acyl amino acid in which hydrogen bonds between peptide-peptide groups and between peptide-carboxylic acid groups have been suggested [6, 18, 19, 20]. However, in the case of *N*-acyl amino acid salt, the interaction through the counter ion needs to be taken into account in addition to the hydrogen bond.

Therefore, in this study the effects of the counter ion size of *N*-acyl amino acid surfactants on the chiral discrimination were investigated through differential

scanning calorimetry (DSC) measurement of the KT and the enthalpy of solution. Additionally, we also studied their interaction in solid states by using Fourier transform infrared spectroscopy (FT-IR) measurements and theoretical calculations. FT-IR spectroscopy is one of the most powerful methods for analysis of the interactions of the peptide group of the *N*-acyl amino acid surfactant [21]. These are discussed from the viewpoint of interactions between peptide-peptide groups and between a peptide group and a counter ion.

Materials and methods

Materials L-Ala, L-Val, L-Phe and their racemic mixtures (DL-form) were purchased from Peptide Institute and Nacalai Tesque, and used without further purification. *N*-Acyl amino acids (C*n*-amino acid; $n = 14, 16$) were synthesized by the reaction of an amino acid with hexadecanoyl or tetradecanoyl chlorides as described previously [22], and were re-crystallized from mixtures of either diethyl ether-ethanol or acetone-methanol. Their purities were checked by HPLC and DSC, and by observing no minimum on surface tension versus concentration curves at 298.15 K. *N*-Acyl amino acids were dissolved in 1 mM excess aqueous solution of alkali hydroxide (LiOH, NaOH, KOH, and CsOH) in order to prevent their precipitation.

DSC analysis DSC experiments were undertaken using a DSC7 (Perkin-Elmer) thermal analyzer. A sample solution was prepared by dissolving *N*-hexadecanoyl amino acids in an aqueous solution of 0.4 M chloride salt, which was sealed in a Large Volume Capsule (Perkin-Elmer) using an O-ring sealed 60 µl stainless steel container. The addition of chloride salt was necessary in order to raise the KT above room temperature for almost all surfactants. The solutions were kept at 2 °C for a day to deposit the solid *N*-hexadecanoyl amino acid salts. The experiments were done using solutions in a concentration range of 10–20 mM using about 50 mg of the samples. The samples were kept at 0 °C for 5 h, and then heated at a rate of 0.5 K/min. At least two runs were performed for each system.

FT-IR analysis Infrared absorption spectra were measured on a JASCO 460 Plus spectrometer by the KBr disc method. Salt samples were prepared by filtering the salts deposited from the alkali solutions of *N*-hexadecanoyl amino acid at 2 °C, and dried in vacuo at 40 °C for a day before measurement. Sixteen scans were accumulated at a resolution of 1 cm^{-1}.

Results and Discussion

DSC analysis

The thermograms of 15 mM aqueous solution of *N*-hexadecanoyl alaninate and valinate surfactants in the presence of the solid deposit are shown in Fig. 1. It can be seen that an endothermic peak accompanies the dissolution of surfactants around the KT, which can be defined as the temperature corresponding to the onset of the peak; these values are shown in Table 1. It was confirmed that the KT values determined by this method are similar

Table 1 Krafft temperatures of the surfactants

Counter ion	Krafft temperature K					
	C16-Ala		C16-Val		C14-Phe	
	L	DL	L	DL	L	DL
Li^+	312.0	330.8	343.4	358.0	305.2	301.1
Na^+	313.9	327.4	306.2	295.7	292.8	304.4
K^+	309.2	302.5	-	-	290.3	298.4
Cs^+	-	-	-	-	284.3	291.8

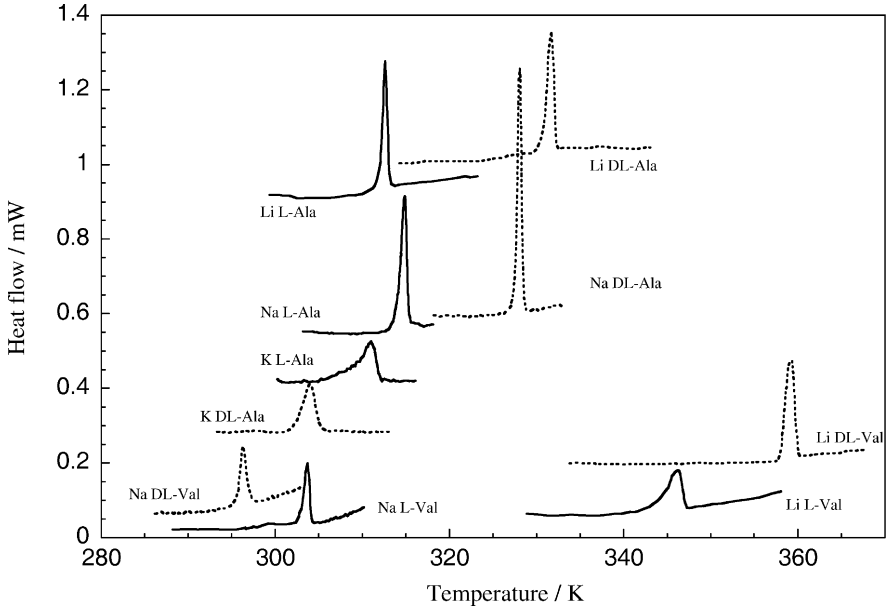


Fig. 1 Thermograms of 15mM aqueous solutions of *N*-hexadecanoyl alaninate and valinate salts. *Solid line* L-form, *dotted line* DL-form

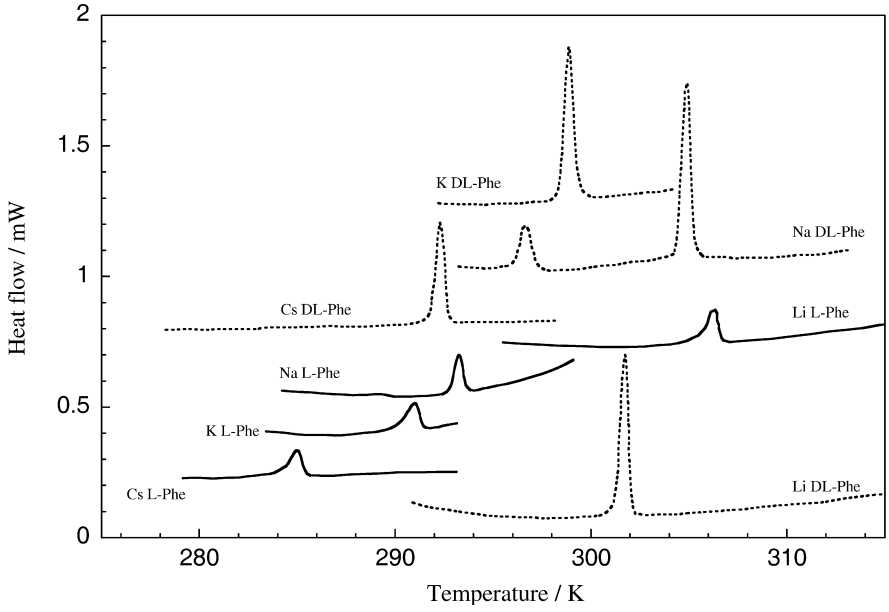


Fig. 2 Thermograms of 15mM aqueous solutions of *N*-tetradecanoyl phenylalaninate salts. *Solid line* L-form, *dotted line* DL-form

to those obtained from the solubility measurements [10]. The thermograms of 15 mM aqueous solutions of *N*-tetradecanoyl phenylalaninate surfactants are shown in Fig. 2. The enthalpy of solution was calculated from the peak area and is shown in Table 2.

Krafft temperature It is seen that the KT of *N*-acyl amino acid surfactants increased with the decreasing size of the ion radius of counter ions except for the partial cases of the Li C16-L-Ala and Li C14-DL-Phe systems. This could be interpreted as the decrease in size of the counter ion making the molecular packing of the surfactant tighten, and the salting out effect enlarge. In particular, the gap of the KT value of C16-Val between Li and Na salts is surprisingly large, being more than 60 K in the case of C16-DL-Val. It is also seen that another peak appears at a lower temperature (269.2 K) than KT in the case of Na C14-DL-Phe; probably caused by a transition in the solid state. The transition enthalpy was 15.4 kJ mol^{-1}.

The chiral effect of the amino acid can be divided into two types: first, where the KT of the DL-form is higher than that of the L-form, and second, where the opposite is the case. In the former, the heterochiral interaction is favored, while in the latter case the homochiral interaction is. An important point to emphasize is that the favorable interaction is shifted from heterochiral to homochiral with an increase in the counter ion radius for both C16-Ala and C16-Val. Figure 3 summarizes the trend in chiral interaction between the isomers of *N*-acyl amino acid surfactant with a higher KT in terms of both sizes of counter ions and the amino acid residue. Here, the case of H^+ as a counter ion was judged by the melting point of *N*-acyl amino acid [10]. This result suggests that the formation of a molecular pair between the L- and D-forms of the *N*-acyl amino acid surfactant is very effective in enhancing the molecular packing in the solid state, with decreasing size of the hydrophilic moiety. From the viewpoint of counter ion size as well as residue size, Phe, which contains an aromatic ring, can be considered an exceptional case. As mentioned in our earlier study [10], interactions between benzyl groups are favorable and are enhanced by a combination of the L- and D-forms. It is concluded that the heterochiral interaction in the *N*-acyl Phe system is extraordinarily strong, and remains favorable even when the counter ion is replaced by a large one such as Cs^+. However, in

Table 2 Enthalpies of solution of the surfactants

Counter ion	Δh_{sol} kJ mol^{-1}					
	C16-Ala		C16-Val		C14-Phe	
	L	DL	L	DL	L	DL
Li^+	40.1 ± 4.1	52.2 ± 3.6	40.1 ± 2.1	56.4 ± 2.9	16.8 ± 1.5	50.0 ± 0.5
Na^+	48.8 ± 2.1	61.8 ± 3.3	41.0 ± 1.5	38.1 ± 2.1	14.9 ± 1.1	61.9 ± 2.1 (15.4)[a]
K^+	35.5 ± 3.8	36.2 ± 2.4	-	-	15.3 ± 0.8	55.9 ± 1.7
Cs^+	-	-	-	-	14.9 ± 0.9	35.3 ± 1.9

[a]Transition enthalpy in solid state at 296.1 K

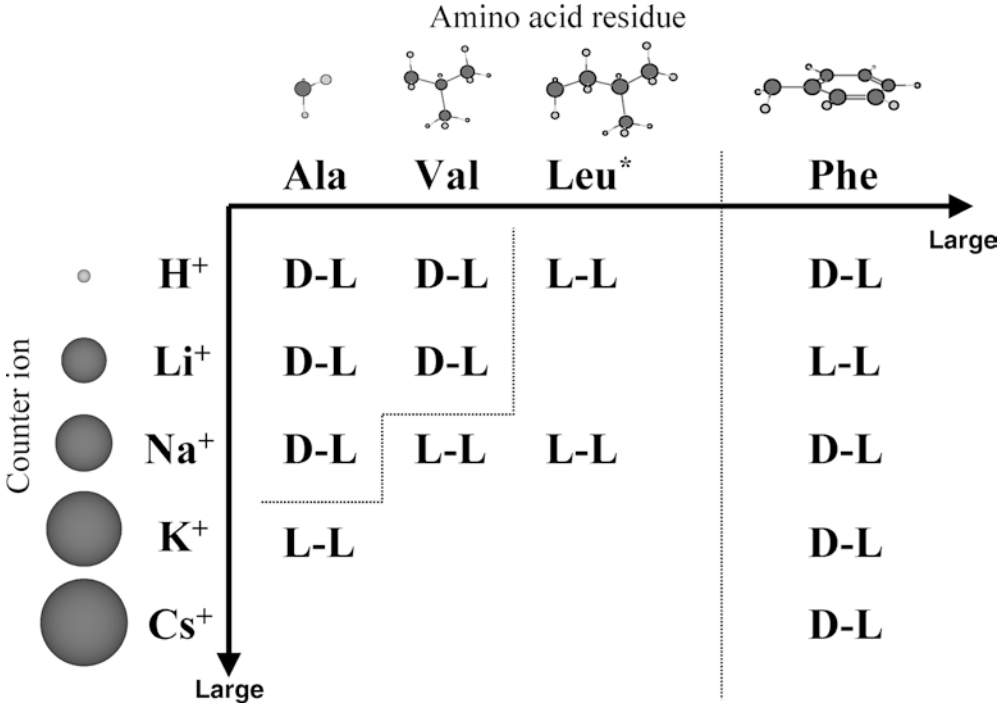


Fig. 3 Schematic diagrams of the effects of the sizes of counter ions and of the amino acid residue on the chiral discrimination for the Krafft temperature (KT) of *N*-acyl amino acid salts [10]

the case of the Li C14-Phe system, the KT of the L-form was higher than that of the DL-form.

Enthalpy of solution The enthalpy of solution, Δh_{sol}, obtained in this study corresponds to the difference between the enthalpy of a surfactant in the micellar state h(micelle) and that in the solid state h(solid).

$$\Delta h_{sol} = h(\text{micelle}) - h(\text{solid}) \quad (1)$$

There are two effects from the increase in size of the counter ion: one is the loosening of molecular packing in the solid state, and the other is the reduction of hydration of the counter ion in the micellar state. These effects raise the values of h(solid) and h(micelle), respectively. From Table 2 we see that the Δh_{sol} value of the Na salt in C16-Ala systems is the largest, irrespective of chirality. Therefore, the difference in Δh_{sol} between Na and K salts is principally attributable to the value of h(solid), while that between Na and Li salts is caused by the variance of h(micelle). The Δh_{sol} value of C14-DL-Phe increases with decreasing counter ion size from Cs^+ to Na^+. This is simply because the molecular packing in the solid state is tightened by the decrease in the counter ion size. In the C16-L-Val and C14-L-Phe systems, however, the Δh_{sol} value is not much affected by the size of the counter ion, and it is suggested that the counter ion size effect contributes equally to both h(solid) and h(micelle).

Next, we examine the chiral effect of an amino acid on the enthalpy of solution. When the KT of the DL-form was higher than that of the L-form, the Δh_{sol} of the DL-form was much larger than that of the L-form. Conversely for the case of Li C14-Phe, the Δh_{sol} value of the DL-form was much larger than that of the L-form, though the KT of the DL-form was lower than that of the L-form. On the other hand, for K C16-Ala and Na C16-Val systems, which are cases where the KT of the DL-form was lower than that of the L-form, there were no remarkable differences between the L- and DL-forms in the value of the Δh_{sol}. Since the racemic effect is very small in a micelle [22], the enthalpy of formation of a racemic compound Δh_{rac} could be evaluated by subtracting the Δh_{sol} value of the DL-form from that of the L-form, under the condition that the temperature dependence of enthalpy can be ignored.

$$\begin{aligned}\Delta h_{rac} &= h_{DL}(\text{solid}) - \frac{1}{2}[h_L(\text{solid}) + h_D(\text{solid})] \\ &= h_{DL}(\text{solid}) - h_L(\text{solid}) = \Delta h_{sol,L} - \Delta h_{sol,DL}\end{aligned} \quad (2)$$

The values obtained for Δh_{rac} of C16-Ala, Li C16-Val, and C14-Phe were ca. -13 kJ mol^{-1}, -16 kJ mol^{-1}, and -20 to -47 kJ mol^{-1}, respectively. These values are much larger than the interaction energies between either methyl or benzyl groups, and correspond to a larger interaction energy, such as with hydrogen bonding or coordination. As mentioned in our previous study, it is quite likely that the combination of the L- and D-forms in those systems enhances the contribution of the coordination of carbonyl or carboxyl groups to alkali metal ions, as well as the contribution of hydrogen bonding between adjacent peptide groups [10]. This question is considered below. The Δh_{rac} value of Li C16-Ala is

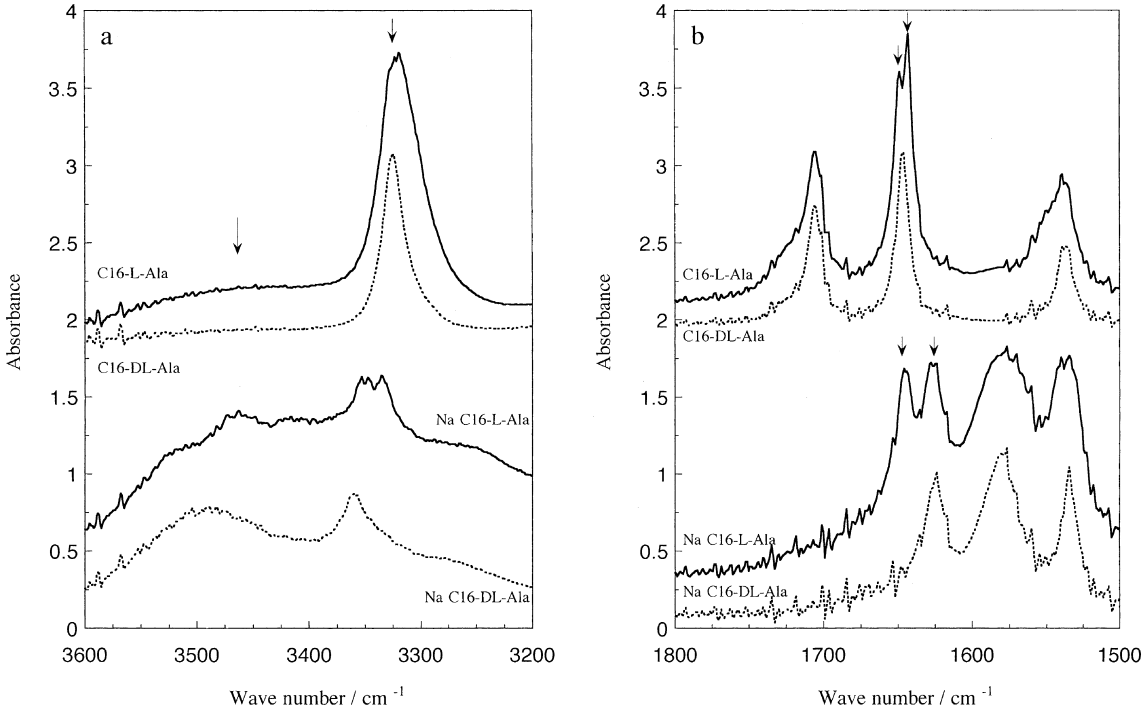


Fig. 4a, b FT-IR spectra of *N*-hexadecanoyl alanine and sodium *N*-hexadecanoyl alaninate. **a** 3,600–3,200 cm^{-1}. **b** 1,800–1,500 cm^{-1}. *1* C16-Ala(acid), *2* Na C16-Ala(salt), *solid line* L-form, *dotted line* DL-form

almost the same as that of Na C16-Ala, while the absolute value of Δh_{rac} of the C14-Phe system increases with decreasing counter ion size from Cs^+ to Na^+. Judging from both the Δh_{rac} and the KT values of Li C14-Phe systems, the solid of Li C14-DL-Phe is a racemic compound and has much smaller entropy than that of Li C14-L-Phe.

IR spectroscopy

Figure 4a shows FT-IR spectra of C16-Ala (acid) and Na C16-Ala (salt) in the solid state in the region of 3,600–3,200 cm^{-1} where the key band is the N-H stretching vibration of the peptide group. Since the absorption band is shifted to a lower wave number by the hydrogen bonding, the bands around 3,500 cm^{-1} and 3,340 cm^{-1} are assigned the free and the hydrogen-bonded N-H stretching vibrations, respectively. From Fig. 4a it can be seen that the absorption of free N-H was absent for C16-DL-Ala, while the absorption for C16-L-Ala was just slightly detected. This supports the idea that the hydrogen bonding ability is a dominant factor for the difference in physicochemical properties between the L- and DL-forms of *N*-acyl amino acid. However, for Na-C16-Ala in Fig. 4a there is a rather large absorption of free N-H in both the L- and DL-forms. Thus, it is difficult to explain the difference between the L- and DL-forms of Na-C16-Ala in terms of hydrogen bonding abilities. Figure 4b shows the same FT-IR spectra in the region of 1,800–1,500 cm^{-1} where the key band is the C=O stretching vibration of the peptide or carboxyl groups. The absorption band around 1,705 cm^{-1} is assigned the C=O stretching of the carboxyl group, and was shifted to around 1,580 cm^{-1} by its neutralization to sodium carboxylate as shown in Fig. 4b. Without distinguishing between acid and salt systems, the absorption band of the DL-form resembles that of the L-form. However the absorption band of the C=O stretching vibration of the peptide group (amide I), which corresponds to the peak around 1,646 cm^{-1}, precisely reflects the distinction of chirality. In the case of the acid form, there is a singlet peak for the DL-form in contrast to a doublet peak for the L-form. This corresponds to the result in Fig. 4a and suggests that the peptide group of C16-L-Ala has two states: one is a hydrogen-bonded state and another is a non-hydrogen-bonded state. On the other hand, in the case of Na L-Ala, a new absorption band appears at 1,625 cm^{-1} in addition to the band around 1,646 cm^{-1}. Furthermore the band around 1,646 cm^{-1} is completely shifted to 1,623 cm^{-1} in the case of Na DL-Ala. These bands appearing at the lower wave number could be assigned the C=O stretching vibration of the peptide group interacting with sodium ions. From these results it was determined that in the case of Na C16-DL-Ala, which has higher KT and h_{sol} values, the peptide group interacts with sodium ions more efficiently than in the case of Na C16-L-Ala.

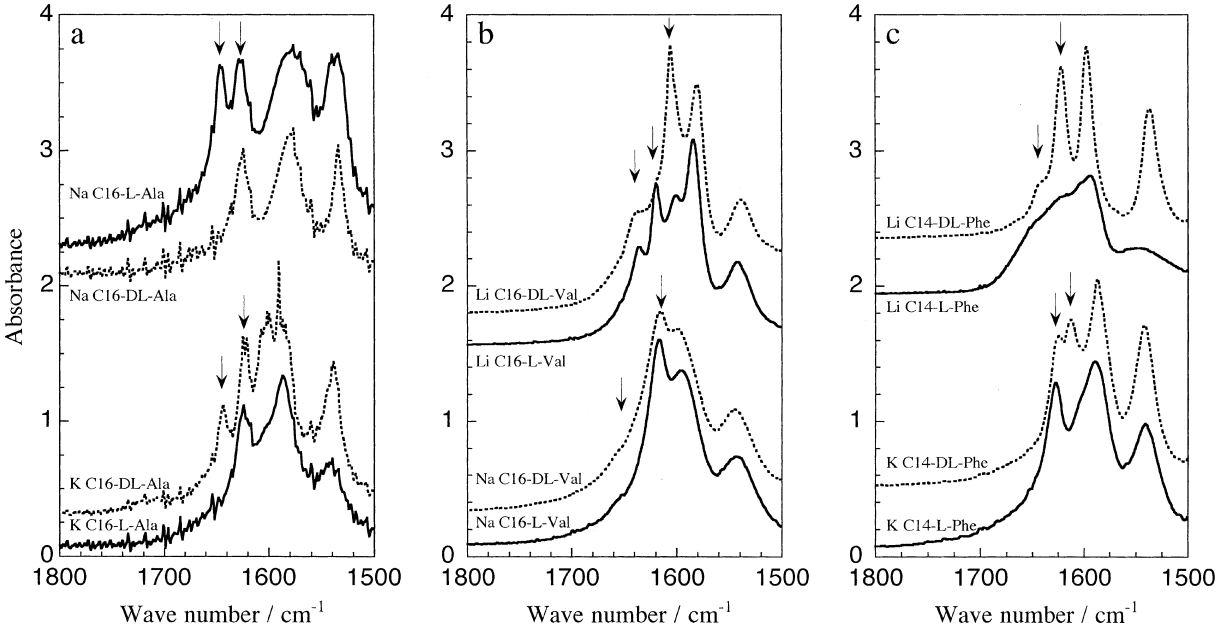
From the above discussion, we can conclude that the observation of the C=O stretching vibration of the peptide group is the most advantageous way to examine the chiral effect of *N*-acyl amino acid salts. Figure 5 shows FT-IR spectra of other samples in the region of 1,800–1,500 cm^{-1}. Interestingly, in the case of the K C16-Ala system, where the DL-form was less stable than the L-form, the band of the C=O stretching vibration of the peptide group of the DL-form only remained around 1,646 cm^{-1}, in contrast to the case of Na C16-Ala (see Fig. 5a). However K C16-DL-Ala was a less-stable racemic compound, because the spectrum of K C16-DL-Ala was different to that of K C16-L-Ala. It can be seen from Fig. 5b that the spectrum of Na C16-DL-Val is almost the same as Na C16-L-Val; this suggests that Na C16-L-Val is not mixed or combined with Na C16-D-Val in the solid state. In Fig. 5b and c are results of Li C16-Val and K C14-Phe, where the DL-form is more stable than the L-form. It was found that the main band of the C=O stretching vibration of the peptide group of the DL-form appeared at a lower wavelength than that of the L-form in all systems. It might be concluded from these results that the difference in the interactions between the peptide group and the alkali metal ion governed chiral discrimination. However, for the Li C14-Phe system where the KT of the DL-form was lower than that of the L-form, the influence of chirality on the spectra was similar to that of the DL-form, which is more stable than the L-form. (see Fig. 5c) This result strongly supports the solid form of Li C14-DL-Phe being a racemic compound. The reason that the KT of the DL-form of Li C14-Phe was lower than that of the L-form appears to be that the free energy of the DL-form is a higher value than the L-form owing to the smaller entropy.

Fig. 5a–c Fourier transform-IR spectra of *N*-acyl amino acid salts. **a** C16-Ala. **b** C16-Val. **c** C14-Phe. *Solid line* L-form, *dotted line* DL-form

Theoretical calculation

In our earlier study [10], we applied *ab initio* calculations to the dimer of sodium *N*-acetyl alaninate (see Fig. 6a) as a model compound to simplify the calculations, and concluded that the difference in the magnitude of the peptide-peptide hydrogen bonding was the dominant factor for the chiral effect. From the FT-IR study, however, it is reasonable to suppose that the dominant factor is the difference in the coordination bonding between the peptide group and the alkali metal ion rather than that of the peptide-peptide hydrogen bonding. Therefore we must reconsider the obtained geometries from this standpoint. The optimized geometries were calculated using the restricted Hartree-Fock procedure with the 6–31+G* basis set. All calculations were performed using Gaussian 98 [23].

The top views of obtained geometries of the L-L and D-L dimers are shown in Fig. 6b. The top views of the two structures seem to be similar, however, the difference is clear from the side view of them in Fig. 6c. In the D-L dimer, each Ala residue is situated in the *trans* position through the bonding between carboxylates, while it is in the *gauche* position for the L-L dimer. Furthermore, it was found that the dimers could interact with the two adjacent molecules at the two coordination bond lines. It should also be noted that one line is parallel to the other in the D-L dimer, whereas the two lines



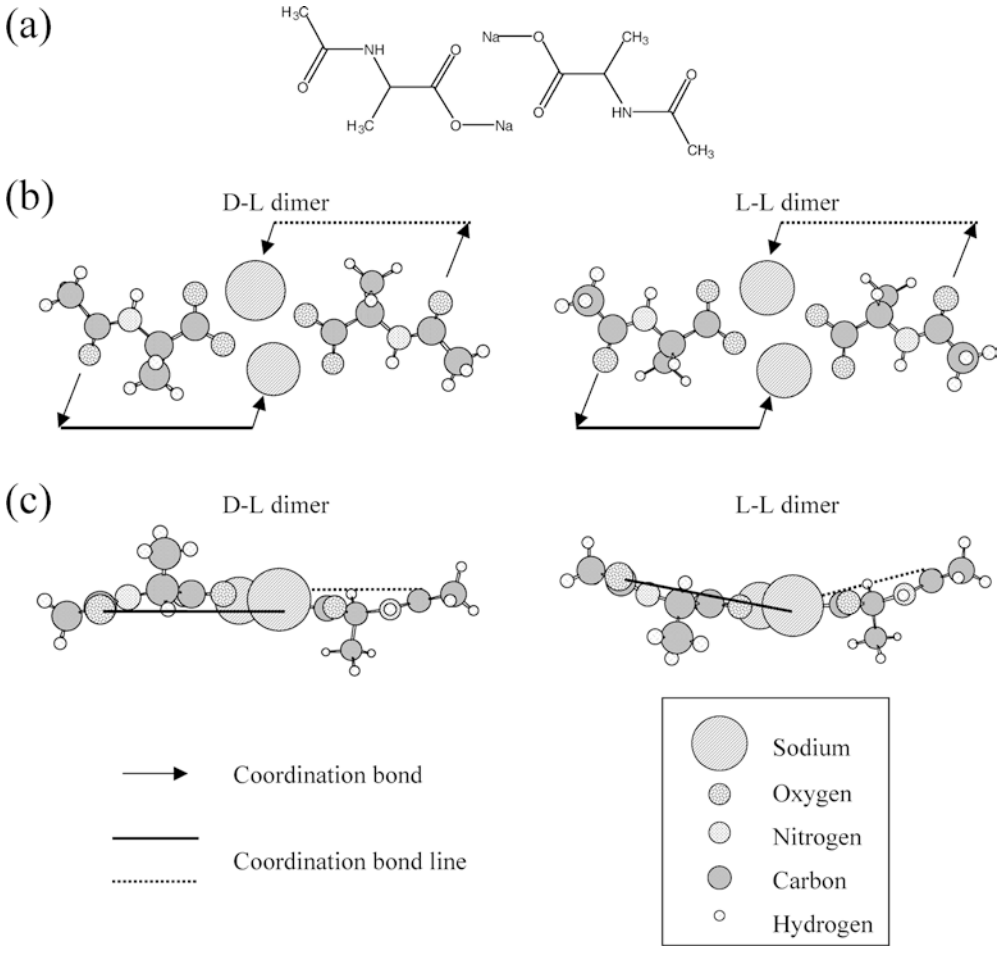


Fig. 6 **a** Chemical structure of dimer of sodium *N*-acetyl alaninate. **b** Top view of optimized dimer geometries. **c** Side view of optimized dimer geometries

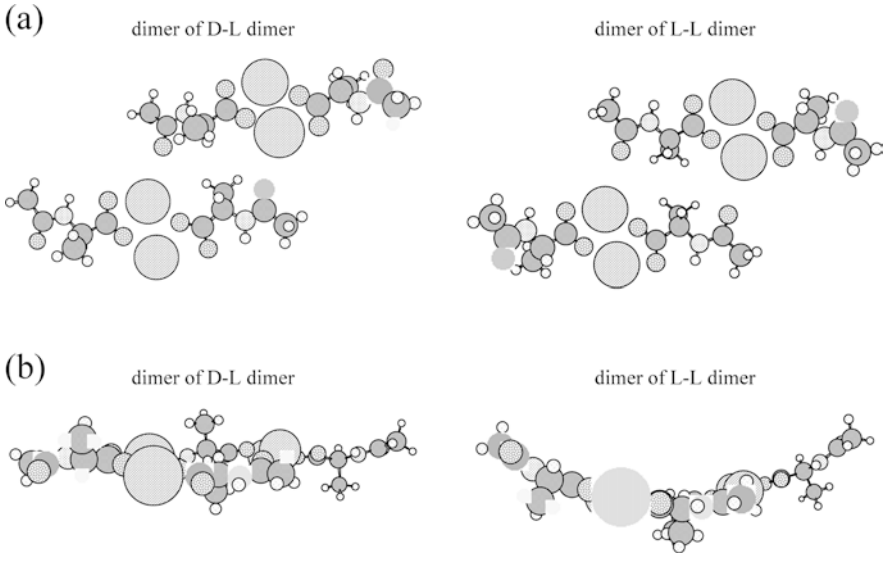


Fig. 7 **a** Top views of optimized geometries of dimer of dimer of sodium *N*-acetyl alaninate. **b** Side views of optimized geometries

intersect each other in the L-L dimer (Fig. 6c). This suggests that the DL form of Na *N*-acyl alaninate can create a sheet structure stabilized by interaction between the peptide group and the sodium ion more effectively than can the L-form. To further substantiate this assumption, semi-empirical MO calculations were

applied to the dimer of the dimer obtained by the *ab initio* calculations. These calculations were performed using MOPAC 2000 with PM3 Hamiltonian [24] under the condition that the geometries of dimers by *ab initio* calculation were fixed. Obtained geometries of the dimer of the DL- and LL-dimers are shown in Fig. 7: it can be seen that the C=O group of peptides did not interact with the N-H group of peptides, but with sodium ions in both cases. Furthermore the axes of the two dimers intersected each other in the case of the LL-dimer, while, as expected, there were two dimers on an almost identical plane in the case of the DL-dimer. Therefore, Na *N*-acyl DL-alaninate becomes a racemic compound in the solid state. This could explain why Na *N*-acyl DL-valinate and K *N*-acyl DL-alaninate are less stable than their L-form counterparts, since the larger hydrophobic residues and counter ions may hinder stacking in the sheet-like structures. Conversely, in the case of Na *N*-acyl DL-phenylalaninate, contact between the benzyl groups in the sheet structure is an advantageous factor and results in the racemic compound.

In summary, the Krafft temperature of *N*-acyl amino acid surfactant increased with decreasing size of the counter ion, except for the cases of Li C16-L-Ala and Li C14-DL-Phe. Experimental results showed that the L-L interaction became superior to the D-L interaction in the solid state of *N*-acyl amino acid surfactant salt for the Ala and Val systems when the size of the counter ion size increased, while the D-L interaction was still advantageous for the Phe system if the counter ion was replaced by a large one such as Cs^+. It was suggested that both interaction between the peptide and the counter ion, and steric hindrance of the amino acid residue govern the chiral effect.

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