Formation of Chiral Aggregates of Acylamino Acids in Solutions

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An alcoholic solution of optically active acylamino acid (AAA) was found to show a circular dichroism (CD) band at 212 nm. The CD band should be inherent to the solution system, since AAA and alcohol *per se* have no absorption band at this wavelength. The CD band intensity decreased on addition of urea and rise in temperature. This is attributed to the formation of chiral aggregate through hydrogen bonds among AAA molecules. Hydrophobic interaction seems to be of minor importance for the formation of the chiral aggregate in alcoholic solution, since the variation of the chain length in the acyl group or addition of myristic acid did not affect the magnitude of profile of the CD band. In aqueous solutions, salts of optically active AAA formed micelles with hydrophobic interactions among the molecules, and only in the case of optically active monosodium salt of *N*-acylglutamic acid (AGS) showed a CD band at 220 nm resulting from the formation of chiral hydrogen bonds at concentrations above the critical micelle concentration (CMC). This type of chiral aggregation may be caused by the existence of free carboxyl group in the optically active AGS molecule.

New lyotropic cholesteric liquid crystals consisting of *N*-acyl-L-glutamic acid (L-AGA) and aromatic solvents were found recently,[1] and a discussion was given on the origin of the circular dichroism (CD) and the iridescent color observed in these liquid crystals.[2] It was deduced that optically active acylamino acids (AAA's) can form lyotropic cholesteric liquid crystals when they are suspended in the solvents in which the acids do not dissolve but undergo swelling. During the course of swelling in some solvents, the optically active AAA molecules are oriented to form a helix.

On the other hand, when L-AGA was completely dissolved in methanol, no iridescent color or CD band around 300—400 nm was observed. However, a new CD band around 200—250 nm was observed for the isotropic solution. Since L-LGA and methanol *per se* have no absorption in this wavelength region, the new CD band should be inherent to the solution systems. The CD band indicates the formation of a chiral aggregate of L-AGA molecules in alcoholic solution, which might result from the asymmetrical interaction between molecules and have a correlation with the formation of cholesteric liquid crystals of L-AGA.

AGA is somewhat similar to protein, having an acyl group neighboring an asymmetric carbon of amino acid and being sufficiently amphiphilic to interact with both hydrophobic and hydrophilic surroundings. Thus an investigation of the chiral aggregate of AGA would be of interest as regards the structure and function of protein.

The function of protein depends on its stereochemical structure, the local structure of proteins, such as α-helix or β-sheet, being determined essentially by their sensitivity toward molecular environments. Some local structures exhibit optical activity similar to that of AAA aggregates by the formation of chiral hydrogen bonding between amino acid residues.

Tachibana and Oda showed that the chirality of the amino acid residue in the polyglutamate determines the asymmetric structure of higher ordered aggregates such as fibrous aggregates[3] and cholesteric films.[4] Saeva and Olin reported that the helical sense of the cholesteric liquid crystals consisting of polyglutamate also depends on the chirality of glutamic acid residue.[5] The higher ordered structures of polyglutamate also result from the hydrogen bond formation among chiral amino acid residues. AGA forms higher ordered chiral structures such as cholesteric liquid crystals[1,2] and chiral aggregates, similar to polyglutamates. They might result from the hydrogen bond formation among molecules since AGA has the same glutamic acid residue which can form hydrogen bond as in polyglutamates. Parthasarthy *et al.* reported the formation of a hydrogen bond among AAA molecules in crystals by means of X-ray diffraction but gave no discussion in view of chirality.[6—8]

We have analysed the CD spectrum of the aggregates of optically active acylamino acids (AAA's) and their salts in isotropic reaction in terms of chiral hydrogen bonding. The results are given in this paper.

Experimental

The AAA's we used are the same as those reported by Takehara *et al.*[9] Their sodium salts can be prepared through neutralization of the corresponding AAA's by the addition of an equivalent quantity of sodium hydroxide.[10] The CD spectra of samples were recorded on a JASCO J-20A spectropolarimeter. Spectral measurements were carried out at constant temperature, the temperature being adjusted by circulation of thermostated water. Solvents used were of spectral grade and used without further purification.

Results and Discussion

Formation of Chiral Aggregate in Alcoholic Solutions. Optically active *N*-lauroylglutamic acid (L- or D-LGA) dissolved in methanol shows a CD band at 212 nm (Fig. 1). The CD band should be inherent to the mixture, since LGA and methanol have no absorption at around 212 nm. L-Enantiomer (L-LGA) and D-enantiomer (D-LGA) have opposite signs and their CD bands should thus be due to the different chiral configuration of LGA. CD bands were also obtained for L-LGA in other solvents such as 1-propanol or 1-octanol, in which L-LGA was apparently completely dissolved (Table 1). Similar CD bands around 212 nm were

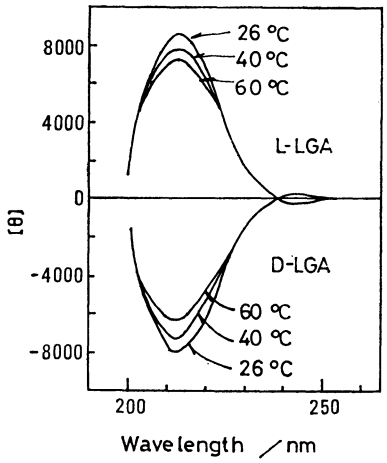

Fig. 1. The CD spectra of optically active *N*-lauroyl-glutamic acid (LGA) in methanol; concentration is 1.0×10^{-2} M.

TABLE 1. EFFECT OF SOLVENTS OR ADDITIVES ON $[\theta]_{212}$ OF L-LGA, 6.1×10^{-3} M, AT 26° C

Solvent	Additive		$[\theta]_{212}$
Methanol	—		8.9×10^3
	Urea	6.0×10^{-1} M	6.9
	Myristic acid	8.8×10^{-3} M	9.2
1-Propanol	—		9.3
1-Octanol	—		8.8

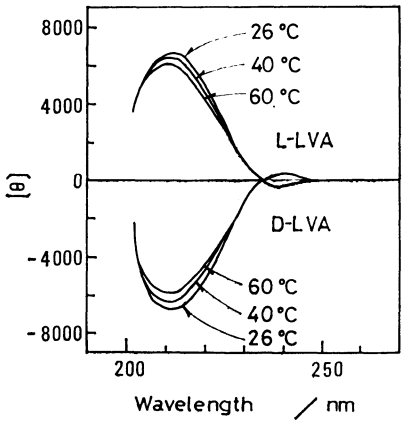

Fig. 2. The CD spectra of optically active *N*-lauroyl-valine (LVA) in methanol; concentration is 1.0×10^{-2} M.

observed in the alcoholic solutions of optically active *N*-lauroylvaline (L- or D-LVA) (Fig. 2). They differ entirely from those of the lyotropic cholesteric liquid crystals consisting of optically active AAA's and solvents,[2] since the wavelengths of the CD band maxima and the spectral profiles differ a great deal in the two cases.[11] The alcoholic solutions showed no birefringence toward the polarized light. The CD bands at 212 nm disappeared at a concentration below 1×10^{-4}, indicating a chiral structure through aggregation. We therefore examined in detail the concentration effect on the CD bands of LGA in alcohols.

Effect of Concentration. The magnitude of the CD band at 212 nm was found to be independent of the concentration of L-LGA or L-LVA in methanol at concentrations higher than 1×10^{-4} M (Fig. 3). This indicates that the number of molecules per aggregate is independent of the concentration of AAA. A concentrated solution of L-LGA (>*ca.* 1×10^{-1} M) in methanol showed birefringence together with a CD band at 212 nm due to the formation of liquid crystaline phase.[12]

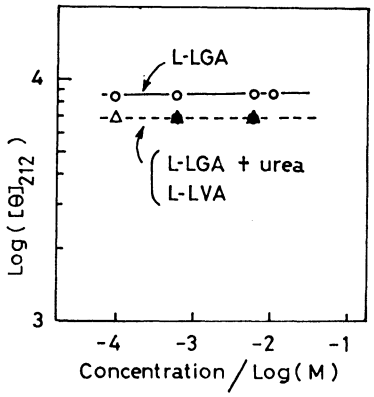

Fig. 3. Relation between concentration of *N*-acyl-L-amino acid and the magnitude of CD in methanol solution at 26 °C.
○: L-LGA, ●: L-LGA+0.6 M urea, △: L-LVA.

Since alcohols have higher affinity to both hydrophobic and hydrophilic parts in the solute molecule, most amphiphilic substances can not form any micelle in alcohols. AAA's have amphiphilic structure in themselves, and the aggregates of AAA's in alcohols do not seem to be micelle.

TABLE 2. $[\theta]_{212}$ IN METHANOL SOLUTION OF *N*-ACYL-L-AMINO ACIDS (L-AAA), 1×10^{-2} M, AT 26 °C

L-AAA	Amino acid residue	Carbon number in the acyl group	$[\theta]_{212}$
L-LGA	Glutamic acid	12	8.5×10^3
L-MGA		14	8.5
L-PGA		16	8.0
L-P_3VA	Valine	3	6.7
L-LVA		12	6.7
L-MVA		14	7.9
L-PVA		16	5.9

Hydrophobic Interactions among the Acyl Long Chains. Since AAA has amphiphilic structure, we examined the effect of hydrophobic counterpart in an AAA molecule on the CD spectral profile. In both cases of L-AGA and *N*-acyl-L-valine (L-AVA) the magnitude of CD band at 212 nm ($[\theta]_{212}$) was almost independent of the chain length of acyl groups in each series (Table 2). In the case of L-AVA, chiral aggregation occurred even for *N*-propionyl-L-valine which seems to have a too short acyl chain to form micelle by the hydrophobic interaction between its acyl side chains. Addition of myristic acid to the methanol solution of L-LGA, by which

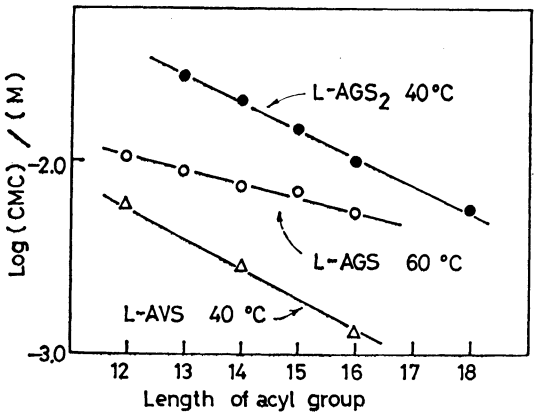

Fig. 4. Relation between log CMC and number of carbon atoms in acyl-group.
○: Monosodium *N*-acyl-L-glutamate (L-AGS),
●: disodium *N*-acyl-L-glutamate (L-AGS$_2$),
△: sodium *N*-acyl-L-valinate (L-AVS).
The CMC data were observed by conductivity method at 60 °C for L-AGS and at 40 °C for L-AGS$_2$ and L-AGS.

perturbation of the hydrophobic interaction between the acyl groups was expected to take place, showed no effect on the value of $[\theta]_{212}$ (Table 1).

The results suggest that hydrophobic interaction is less important for the formation of chiral aggregates. On the other hand, values of critical micelle concentration (CMC) in aqueous solutions of AAA were dependent on the lengths of acyl groups (Fig. 4)[10,13] and hydrophobic interaction was essential for the micelle formation. The structure of the chiral aggregates in alcoholic solutions should be quite different from the micelle structure in aqueous solutions.

Aggregation by Hydrogen Bonding. On addition of urea, which tends to break hydrogen bonds in the proteins,[14] the values of $[\theta]_{212}$ for the alcoholic solutions of AAA decreased (Table 1 and Fig. 3). L-LGA and L-LVA showed no CD band at 212 nm in trifluoroacetic acid, a hydrogen bond breaker stronger than urea. The formation of chiral aggregates of optically active AAA in alcohols may be caused by the hydrogen bond formation between AAA molecules themselves. The decrease in the value of $[\theta]_{212}$ at higher temperatures may be caused by hydrogen bonds rupture in the aggregates (Figs. 1 and 2).

Proteins form second order structure with the hydrogen bond between NH-group and CO-group in the molecule[15] and its helical sense is partially determined by the asymmetric carbon in the amino acid residue. The α-helical conformation, including the hydrogen bonds, results in the appearance of CD bands at 191, 209, and 222 nm.[16] From these properties of proteins, it was assumed that the CD band at 212 nm in alcoholic solutions of optically active AAA indicates the formation of the intermolecular chiral hydrogen bond.

Two possible hydrogen donors in AAA, NH in amide group and OH in carboxyl group, can be considered. The latter may have no contribution to the hydrogen bond formation, since a similar CD band at 212 nm was observed in the methanol solution of

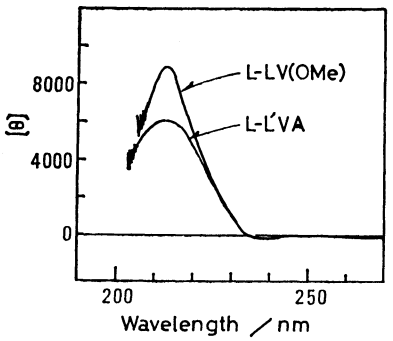

Fig. 5. CD spectra of *N*-lauryl-L-valine (L-L'VA) and methyl ester of *N*-lauroyl-L-valine(L-LV(OMe)) in methanol; at 40 °C and 1.0×10^{-2} M.

methyl ester of L-LVA (Fig. 5). We can also expect two types of hydrogen acceptor in AAA, amide carbonyl and carbonyl of carboxylic group. CO in the amide group may have no participation for the hydrogen bond formation, since the CD band at 212 nm could not be detected for the methanol solution of *N*-lauryl-L-valine(L-L'VA), an *N*-alkylated amino acid without amide group (Fig. 5). An intermolecular chiral hydrogen bond may occur between NH in amide group and CO in carboxyl group of AAA. Mutual orientation between the NH and CO groups is determined by the configuration of the asymmetric carbon being adjacent to both. In the case of racemic mixture of AAA in methanol, no CD band could be detected. Thus, the aggregation of optically active AAA in alcoholic solution occurrs through the chiral hydrogen bonds among AAA molecules.

Table 3. Critical micelle concentration (CMC) of monosodium *N*-acyl-L-glutamate (L-AGS) and disodium *N*-acyl-L-glutamate (L-AGS$_2$)

	Carbon number in the acyl group	CMC/mM	
		By conductivity method[10]	By CD method
L-AGS	12	10.6[a]	9[a]
	14	7.2[b]	8[b]
	16	5—6[b]	5[b]
L-AGS$_2$	14	21[a]	—
	16	9.8[a]	—

a) At 40 °C. b) At 60 °C.

CD of Optically Active AAA in Aqueous Solution. The sodium salts of optically active AAA's form micelles in aqueous solutions at concentrations above their CMC (Table 3).[10] If the chiral aggregation occurrs together with micelle formation, the existence of a CD band and its concentration dependence are expected for the aqueous solutions of the sodium salts of optically active AAA's. Only monosodium salt of *N*-acylglutamic acid (L- or D-AGA) shows a CD band at 220 nm (Fig. 6). The concentration dependency of this CD magnitude was found to be correlated with its CMC (Fig. 7 and Table 3). For example, the magnitude of CD band at 220 nm, $[\theta]_{220}$, observed for the aqueous solution of monosodium salt of *N*-lauroyl-L-glutamic acid (L-LGS)

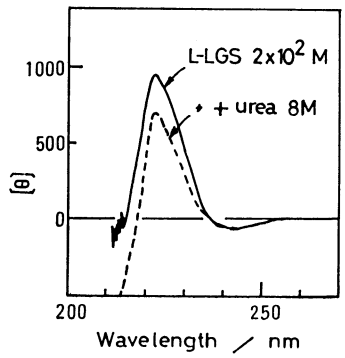

Fig. 6. The CD spectra of monosodium *N*-lauroyl-L-glutamate (L-LGS) in water at 40 °C.
Solid line: L-LGS, 2×10^{-2} M.
Broken line: L-LGS, 2×10^{-2} M and urea, 8 M.

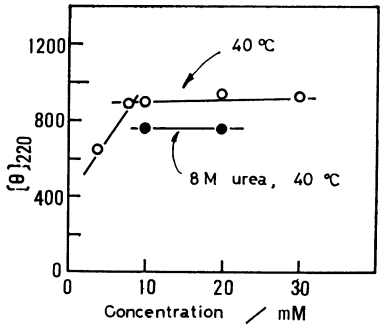

Fig. 7. Relation between $[\theta]_{220}$ and concentration of monosodium *N*-lauroyl-L-glutamate(L-LGS) in water, at 40 °C.

increased with increase in the concentration of L-LGS upto about 1×10^{-2} M which corresponds to its CMC. At concentrations above 1×10^{-2} M, $[\theta]_{220}$ was approximately constant (Fig. 7). Addition of urea to the aqueous solution of L-LGS decreased the CD magnitude in a similar way to that in the case of alcoholic solution (Figs. 4, 6, and 7). Thus, the CD band at 220 nm observed for optically active AGS in aqueous solutions suggests the occurrence of micelle formation together with the formation of chiral hydrogen bond.

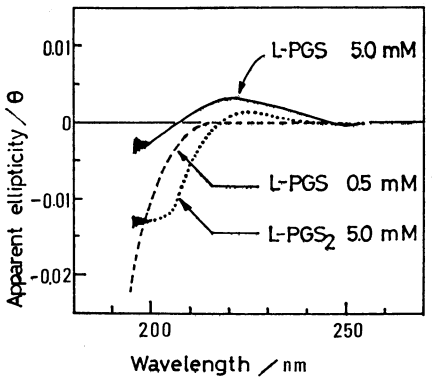

Fig. 8. The CD spectra of sodium and disodium *N*-palmitoyl-L-glutamate (L-PGS and L-PGS_2).
Solid line: L-PGS, 5.0 mM (above CMC), at 60 °C.
Broken line: L-PGS, 0.5 mM (below CMC), at 60 °C.
Dotted line: L-PGS_2, 5.0 mM (below CMC), at 40 °C.

At concentrations lower than CMC, L-AGS shows another CD band at *ca.* 200 nm with the opposite sign to that of the CD band at *ca.* 220 nm (Fig. 8). On the other hand, the aqueous solutions of optically active disodium *N*-acylglutamates (L- or D-AGS_2) and optically active sodium *N*-acylvalinates (L- or D-AVS) show a CD band only at *ca.* 200 nm irrespective of their concentration. From the results, the CD band at 200 nm is attributable to the asymmetric structure of the optically active AGS, AGS_2, and AVS themselves. Since CMC's of these surfactants are dependent on the length of acyl groups (Fig. 4 and Table 3), the micelle formation might be caused by the hydrophobic interaction among acyl chains. On the surface of micelles, amino acid residues of these surfactants can not be brought close enough to form hydrogen bonds because of the repulsive interaction among the carboxylate anions. In the case of L-AGS the decrease in CMC with increase of the acyl chain length is a little smaller than in the case of L-AGS_2 and L-AVS (Fig. 4). This suggests that the hydrophobic interaction is less in L-AGS than in L-AGS_2 and L-AVS. The mutual repulsion between amino acid residues on the surface of L-AGS micelles might be relaxed by the existence of a free carboxyl group. The free carboxyl group in L-AGS is assumed to form chiral hydrogen bond, which may be correlated to the CD band at 220 nm.

The structure of the aggregates of optically active AAA's or their salts are determined by solvent. In alcoholic solutions, AAA's form a different aggregate from micelle through the chiral hydrogen bonding among AAA molecules. However, in aqueous solutions, salts of optically active AAA's form micelles by the hydrophobic interaction among their side chains; the hydrophobic interaction induces hydrogen bond formation in the parts of the hydrophilic moiety of L-AGS.

Conclusion

A CD band at 212 nm was found in alcoholic solutions of optically active AAA's. Hydrophobic interaction among the long chains of acyl groups of AAA molecules is of minor importance to the formation of their aggregates, since the spectral profile and magnitude of the CD bands are independent of the lengths of the acyl groups in the AAA molecules and of their concentrations. Hydrogen bonds might break under certain conditions. The addition of urea and rise in temperature decrease the magnitude of the CD bands, and thus the aggregate may result from the chiral hydrogen bond formation among AAA molecules.

On the other hand, in aqueous solutions, salts of optically active AAA's form micelles with hydrophobic interaction among the molecuels, and show a CD band at 220 nm at concentrations above its CMC only in the case of optically active AGS. The CD band at 220 nm corresponds to the formation of chiral hydrogen bond caused by the existence of a free carboxyl group.

AAA's have a certain similarity to proteins, having acyl group juxtaposition to the asymmetric carbon and becoming amphiphilic to interact both with hydrophobic and hydrophilic surroundings. Proteins em-

bedded in biological membranes reveal two different features. Inside membranes where hydrophobic environment is predominant, the embedded proteins behave like a polar solute in lipid, being forced to form intramolecular hydrogen bonding inside the proteins. In contrast, at the surface of membranes where hydrophilic environment is predominant, the buried proteins are hydrated and seem to be suppressed to form a chiral hydrogen bond. The chiral aggregation or micelle formation of AAA's, which reflects the interaction of AAA's with environmental solvents, may help us to understand the behavior of proteins embedded in membranes.

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11) The maximum wavelength of CD band attributable to the cholesteric helical structure for the L-LGA–benzene system appears at *ca.* 300—400 nm, its spectral profile being more broad than that of the CD band at 212 nm for the L-LGA–methanol solution.
12) The liquid crystal showed no CD band corresponding to the cholesteric structure, and was assumed to be another type of lyotropic liquid crystal differing from the L-LGA–benzene system.
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