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Studies of Acylamino Acid Monolayers by π-A curves. I. Acylamino Acids with Alkyl Side Chain

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In order to obtain some information regarding the behavior of amino acids at interfaces, the monolayers of *N*-acyl L-α-amino acids with alkyl side chains on water have been investigated by measuring the surface pressure *vs.* area curves at various temperatures. It has been found that *N*-octadecanoylglycine and *N*-octadecanoylalanine form the condensed monolayers over the whole surface pressure range measured, *N*-octadecanoylvaline forms the expanded monolayer at a lower surface pressure, and *N*-octadecanoylleucine and *N*-octadecanoylisoleucine collapse at high surface pressures. From the experimental results, it has been concluded that the amino acid residue with a long side chain occupies a large area at the air/water interface and interferes with the condensation of acylamino acid monolayer. It has also been found that the methyl esterification of amino acid residue makes the monolayer state unstable although it produces less significant effect than the increase in the alkyl chain length of amino acid residue.

The behavior of amino acid molecules at interfaces is of great interest from the biological and chemical viewpoints. The conformation of protein molecules is clearly related to the orientation of the side chains of amino acids and the interaction between them. It is noted that surfactants synthesized from amino acids are important in connection with their biode-composition.

Several investigations were carried out for the surface activity of amino acids and their derivatives.[1–4] The critical micelle concentration of mixtures of *N*-lauroyl amino acid sodium salts was also measured.[5] On the other hand, the antibacterial ability of amino acid derivatives was reported by several workers.[6,7] Now it is necessary to investigate systematically the behavior of amino acid molecules at interfaces. For this purpose, insoluble monolayers of acylamino acids on water seem to be appropriate.

In the present paper, *N*-acyl L-α-amino acids with alkyl side chains are used and the surface pressure *vs.* area curves are measured as a function of temperature.

Experimental

Materials. Eight *N*-acyl L-α-amino acids and two *N*-acyl L-α-amino acid methyl esters used in this work are listed with their abbreviations in Table 1. They were prepared from fatty acids and L-α-amino acids. The amino acids (Wako Pure Chemical Industries, Special Grade) were used without further purification. The fatty acids were purified by repeated recrystallizations from ethyl alcohol. Amino acid methyl ester hydrochlorides were first synthesized by the thionyl chloride method.[7] Next, acylamino acid methyl esters were synthesized by making them react with fatty acids in the presence of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride in chloroform.[8] Acylamino acids were obtained by the hydrolysis of acylamino acid methyl esters. The acylamino acid methyl esters were purified by repeated recrystallizations from methyl alcohol. The acylamino acids were recrystallized from ethyl alcohol and acetone. Their purity was checked by the elemental analysis and potential difference titration.

TABLE 1. MATERIALS USED

Compound	Abbreviation
Octadecanoylglycine	OcD–Gly
Octadecanoylalanine	OcD–Ala
Octadecanoylvaline	OcD–Val
Octadecanoylleucine	OcD–Leu
Octadecanoylisoleucine	OcD–Ile
Docosanoylvaline	Doc–Val
Docosanoylleucine	Doc–Leu
Docosanoylisoleucine	Doc–Ile
Octadecanoylvaline methylester	OcD–Val–OMe
Docosanoylvaline methylester	Doc–Val–OMe

Procedure. Benzene was used as the spreading solvent; for OcD–Gly, a small amount of ethyl alcohol was added to facilitate its dissolution. The surface pressure was measured by a Wilhelmy type surface balance. The films were compressed at the constant rate of 0.017 nm^2 min^{-1}. The temperature was held constant within 0.1 K.

Results and Discussion

It is reasonably expected that the difference in the interfacial behavior between amino acids is reflected in the surface pressure *vs.* area curves of acylamino acid monolayers. First, the surface pressure π of OcD–Gly, OcD–Ala, OcD–Val, OcD–Leu, and OcD–Ile monolayers on water were measured as a function of area A peroctadecanoylamino acid molecule at 298.2 K. The surface pressure *vs.* area curves of acylamino acids are depicted in Fig. 1. It is seen that they differ remarkably with each other and become expanded with increase in the length of the alkyl side chain of amino acid. The π *vs.* A curve of OcD–Gly seems to be quite similar to that of octadecanoic acid. This fact indicates that OcD–Gly forms a condensed film. Therefore, we may say that the glycine residue of OcD–Gly is immersed in the substrate and acts as a part of the hydrophilic portion. The OcD–Ala monolayer also seems to form the condensed film, although its limiting area estimated from the π *vs.* A curve is larger than that of OcD–Gly. This may be attributable to a bigger side chain of the alanine residue.

On the other hand, it is seen from Fig. 1 that the π *vs.* A curves of OcD–Val, OcD–Leu, and OcD–Ile monolayers are significantly different in shape from those of OcD–Gly and OcD–Ala monolayers. The OcD–Val, OcD–Leu, and OcD–Ile monolayers are clearly expanded films in a lower surface pressure region. In a higher surface pressure region, they seem to transform into other states; however, the transformation of OcD-Val is markedly different from those of OcD-Leu and OcD-Ile. In order to clarify this point, the variation of π *vs.* A curve with temperature was investigated.

The π *vs.* A curves of OcD-Val monolayer at 283.2, 288.2, 293.2, 298.2, 300.7, and 303.2 K are shown in Fig. 2. The surface pressure of the break point on the curve is found to increase greatly with increasing temperature. This temperature dependence resembles that of the film-forming substance which transforms from the expanded to the condensed state.[9–11] Furthermore, it turns out that the OcD-Val monolayer forms a condensed film over the whole surface pressure range measured at 283.2 K since its π *vs.* A curve is similar to that of OcD-Ala monolayer shown in Fig. 1. Thus, we can conclude that the OcD-Val monolayer transforms from the expanded to the condensed film at the break of the π *vs.* A curve. Comparing the π *vs.* A curve of OcD-Val monolayer with that of OcD-Ala monolayer, it is noticed that the bigger side chain of valine residue makes its state expanded up to a fairly high surface pressure at 298.2 K.

Similarly, the π *vs.* A curves of OcD-Leu and OcD-Ile, which have longer side chains than OcD-Val, were measured as a function of temperature; their results are illustrated in Figs. 3 and 4, respectively. It is seen that the surface pressure increases slightly with increasing temperature at a large area while it varies irregularly with temperature at a small area. Furthermore, the shape of the curves at the transition region are abnormal. This fact may indicate that the monolayers collapse and change into three-dimensional

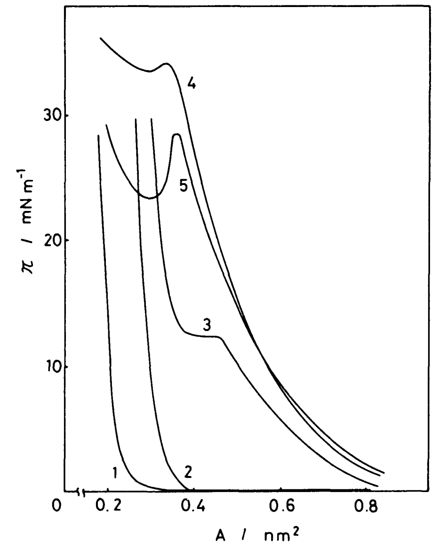

Fig. 1. Surface tension *vs.* area curves of Octadecanoylamino acid monolayers at 298.2 K. 1) OcD-Gly, 2) OcD-Ala, 3) OcD-Val, 4) OcD-Leu, 5) OcD-Ile.

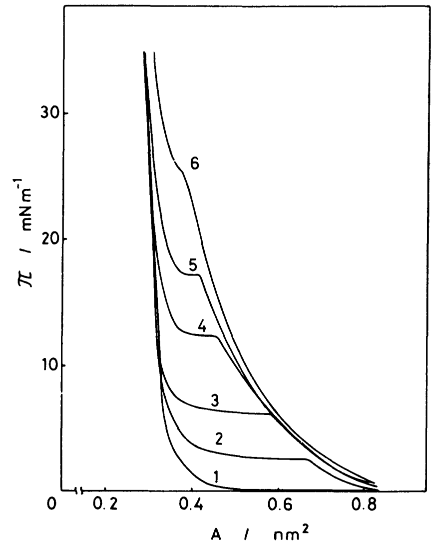

Fig. 2. Surface tension *vs.* area curves of OcD-Val monolayer. 1) 283.2 K, 2) 288.2 K, 3) 293.2 K, 4) 298.2 K, 5) 300.7 K, 6) 303.2 K.

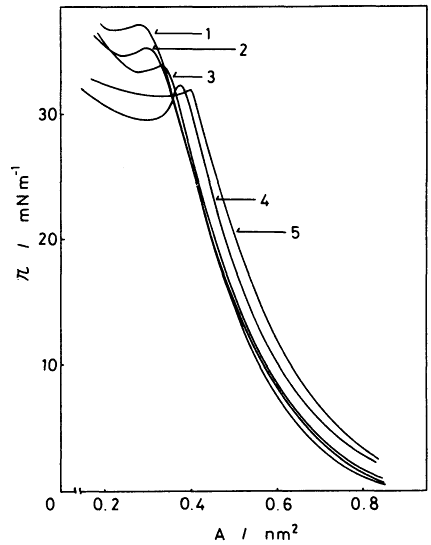

Fig. 3. Surface tension *vs.* area curves of OcD-Leu monolayer. 1) 288.2 K, 2) 293.2 K, 3) 298.2 K, 4) 300.7 K, 5) 303.2 K.

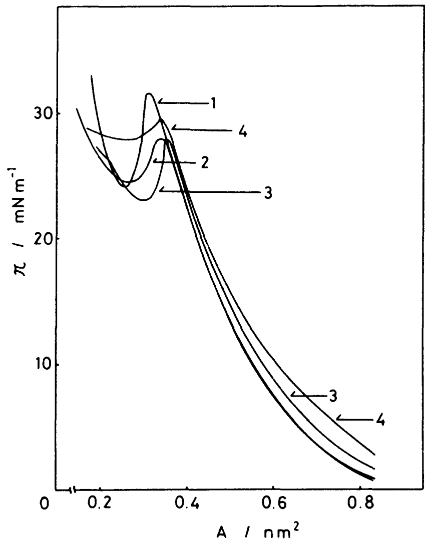

Fig. 4. Surface tension *vs.* area curves of OcD-Ile monolayer. 1) 283.2 K, 2) 293.2 K, 3) 298.2 K, 4) 303.2 K.

bulk phases at high surface pressures. It may be said, therefore, that the intermolecular force between the octadecanoyl groups is not strong enough to make the condensed monolayers of OcD–Leu and OcD–Ile stable. Now measurements seem to be required for acylamino acids having a longer acyl chain.

The π *vs.* A curves of Doc–Leu and Doc–Ile measured at various temperatures are illustrated in Figs. 5 and 6, respectively. It is seen from Fig. 6 that Doc–Ile forms a condensed film of which the limiting area is larger than that of the OcD–Val monolayer at 283.2 K. With an increase in temperature, the Doc–Ile monolayer becomes expanded in a lower surface pressure region. At temperatures above 303.2 K, the curve shows a striking resemblance to that of OcD–Ile given in Fig. 4. In the case of Doc–Leu, the temperature dependence of the π *vs.* A curve is qualitatively similar to that of Doc–Ile, although a typical condensed film is not realized even at the lowest temperature measured. The above results lead us to the conclusion that the amino acid residue with a long side chain occupies a large area at the air/water interface and interferes with the condensation of acylamino acid monolayer. Furthermore, it is noteworthy that the collapse of the expanded films is attended by remarkable super-compression.

It is important to examine the difference between the π *vs.* A curves of *N*-acylleucine and *N*-acylisoleucine which are isomeric with each other. From Figs. 3 and 4, we can see that the OcD–Leu monolayer remains in an expanded state up to a higher surface pressure value than the OcD–Ile monolayer. Comparison between Figs. 5 and 6 tells us that the expanded film of Doc–Leu is hard to transform into the condensed film. Therefore, it can be said that the isobutyl group of *N*-acylleucine forces the monolayer to stay in the expanded state up to a high surface pressure as compared with the s-butyl group of *N*-acylisoleucine.

It is now interesting to investigate the behavior of the monolayer of *N*-acylvaline methyl ester which is an isomer of *N*-acylleucine and *N*-acylisoleucine although different in polar group. In Fig. 7, the π *vs.* A curve of OcD–Val–OMe are drawn at various temperatures. The temperature dependence resembles that of Doc–Leu and Doc–Ile rather than of OcD–Leu and OcD–Ile. It is clear by comparison with Fig. 2 that the methyl esterification makes the monolayer state somewhat unstable. Therefore, Doc–Val–OMe may be expected to form a condensed film at temperatures under consideration by the strong intermolecular interaction between docosanoyl groups. The experimental results for Doc–Val–OMe, which are illustrated in Fig. 8, support the above view. These findings indicate that the increase in the alkyl chain length of the amino acid residue by one carbon atom produces more significant effect on the monolayer behavior than the methyl esterification of the carboxyl group.

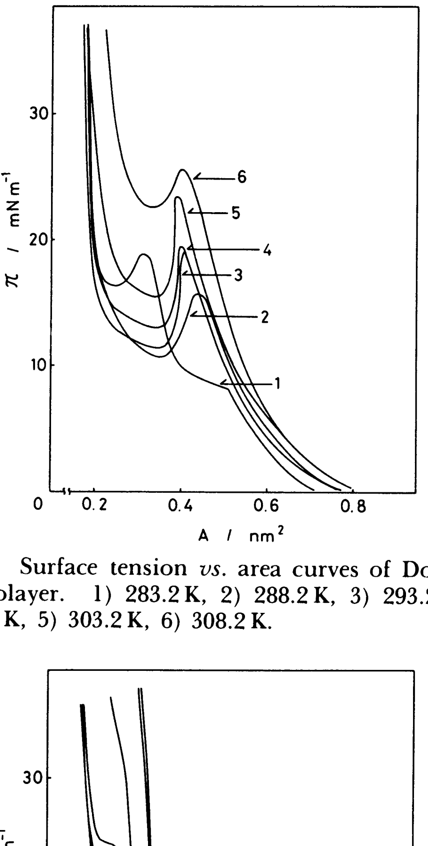

Fig. 5. Surface tension *vs.* area curves of Doc–Leu monolayer. 1) 283.2 K, 2) 288.2 K, 3) 293.2 K, 4) 298.2 K, 5) 303.2 K, 6) 308.2 K.

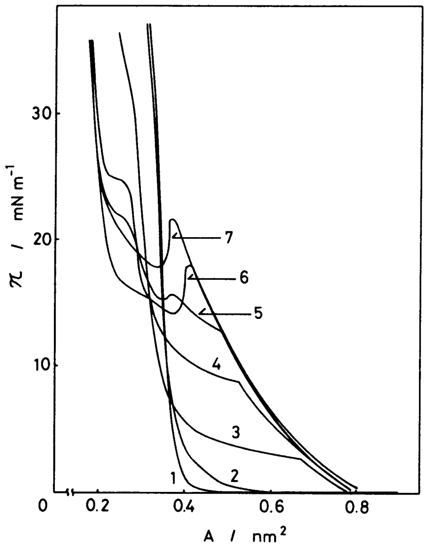

Fig. 6. Surface tension *vs.* area curves of Doc–Ile monolayer. 1) 283.2 K, 2) 288.2 K, 3) 293.2 K, 4) 298.2 K, 5) 300.7 K, 6) 303.2 K, 7) 308.2 K.

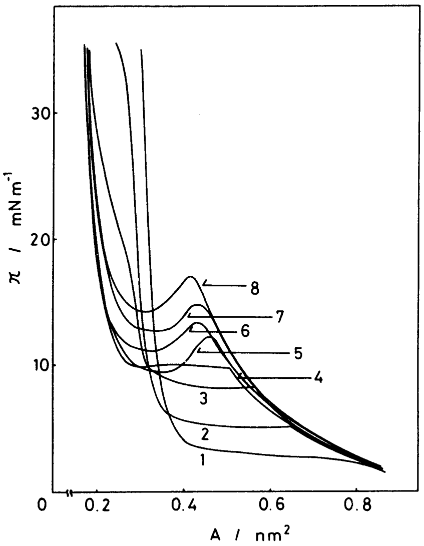

Fig. 7. Surface tension *vs.* area curves of OcD–Val–OMe monolayer. 1) 283.2 K, 2) 288.2 K, 3) 293.2 K, 4) 295.7 K, 5) 298.2 K, 6) 300.7 K, 7) 303.2 K, 8) 308.2 K.

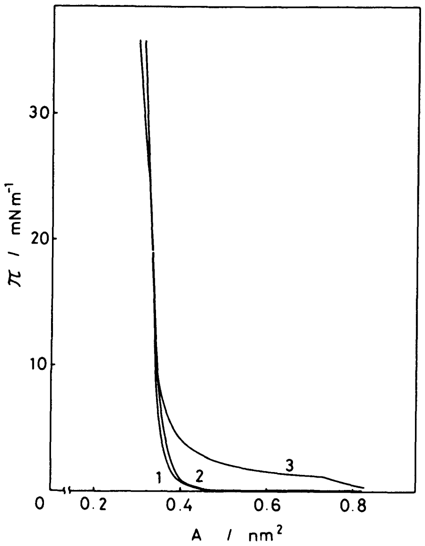

Fig. 8. Surface tension *vs.* area curves of Doc–Val–OMe monolayer. 1) 283.2 K, 2) 303.2 K, 3) 308.2 K.

It has been found from the measurements on the monolayers of acylamino acids that the length and chemical structure of the alkyl side chain of amino acid play a significant role in the monolayer behavior. Further information will be provided by making a similar investigation of amino acids with aromatic or polar side chains.

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