Studies on Long Chain Alkyl Amides of Water-soluble Poly-α-amino Acids. I. Amphipathic Properties of Poly-DL-alanine Dodecylamide

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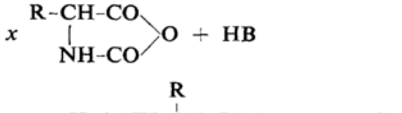
It has been established that poly-α-amino acid can be synthesized by the polymerization of *N*-carboxy-α-amino acid anhydride[1,2], and a basic substance such as sodium hydroxide, sodium methoxide or organic amines (e. g., *n*-hexylamine, triethylamine) initiates the polymerization effectively[1-3]. The polymerization reaction, initiated by a base (HB) capable of generating an active hydrogen, proceeds as a stepwise addition of amino acid residues in the following manner:



The free amino group of poly-α-amino acid produced in each step of the reaction reacts with another molecule of *N*-carboxy anhydride. The stepwise polymerization then goes on until all the *N*-carboxy anhydride molecules are consumed, and the resulting poly-α-amino acid would have a number average degree of polymerization (x) equal to the initial anhydride-initiator molar ratio ([A]/[I]).

The poly-α-amino acid prepared in this way would have a basic residue (B) at the terminal carboxyl group linked by an amide bond, if an organic amine such as primary alkyl amine has been chosen as the initiator for polymerization. Thus an amphipathic poly-α-amino acid amide, which has both hydrophilic and hydrophobic groups in a molecule, may be readily synthesized by the above method, if the following conditions are satisfied. The poly-α-amino acid is soluble in water, the basic residue is composed of a hydrophobic group such as long paraffin chain, and the hydrophility of the poly-α-amino acid part is well balanced with the hydrophobicity of the basic residue, i. e., the initial [A]/[I] ratio is properly chosen.

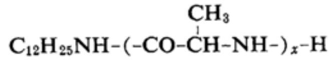
Aside from the difference in chemical structure, the chain conformation and other related properties of poly-α-amino acid are greatly dependent on the constituent amino acid residue[4] as well as on the degree of polymerization, medium and temperature. The manifold possibility in the use of various water-soluble poly-α-amino acids would make the studies of amphipatic poly-α-amino acid alkylamide significant for elucidating the hydrophilic nature of different amino acid residues and the effect of the type of residue on the properties of amphipathic polymer and its hydrogen bonding nature. Furthermore, it is expected that the polymers would find practical uses as surface active agents.

It is noted that the reaction mechanism of polymerization of *N*-carboxy-α-amino acid anhydride is apparently analogous to that of ethylene oxide[5] and the structure of poly-α-amino acid amide resembles that of polyoxyethylene alkyl amine or ether.

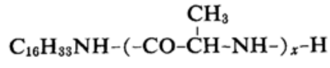
The present paper deals with the synthesis and amphipathic nature of poly-DL-alanine alkyl amide, especially, of poly-DL-alanine dodecylamide, in relation to the degree of polymerization of polyamino acid part in a molecule.

Synthesis

N-Carboxy-DL-alanine anhydride (DL-alanine NCA) was prepared from DL-alanine by the phosgene method[6]. The polymerizations were initiated with either dodecylamine, cetylamine or *N*-myristyl-β-alanine at [A]/[I] ratios which varied from 5 to 30. The polymers obtained were dodecylamide (I), cetylamide (II) or *N*-carboxyethyl-myristylamide (III) of poly-DL-alanine:



$$C_{12}H_{25}NH\text{-}(\text{-}CO\text{-}\overset{CH_3}{\overset{|}{C}H}\text{-}NH\text{-})_x\text{-}H \qquad (I)$$



$$C_{16}H_{33}NH\text{-}(\text{-}CO\text{-}\overset{CH_3}{\overset{|}{C}H}\text{-}NH\text{-})_x\text{-}H \qquad (II)$$

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[1] E. Katchalski and M. Sela, *Adv. Protein Chem.*, **13**, 243 (1958).

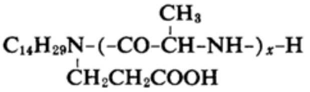
[2] E. Katchalski, ibid., **6**, 123 (1951).

[3] E. R. Blout and R. H. Karlson, *J. Am. Chem. Soc.*, **78**, 941 (1956).

[4] e. g., E. R. Blout, C. de Lozé, S. M. Bloom and G. D. Fasman, ibid., **82**, 3787 (1960).

[5] P. J. Flory, ibid., **62**, 1561 (1940).

[6] A. C. Farthing, *J. Chem. Soc.*, **1950**, 3213; J. L. Bailey, ibid., **1950**, 3461.



$$C_{14}H_{29}N\text{-}(\text{-CO-}\overset{CH_3}{\underset{CH_2CH_2COOH}{CH}}\text{-NH-})_x\text{-H} \qquad \text{(III)}$$

Among them, poly-DL-alanine dodecylamide (I, abbreviated to PADA) was investigated in some detail, and some of its characteristics are listed in Table I, with reference to the [A]/[I] ratio.

TABLE I. CHARACTERISTICS OF PADA AND POLY-DL-ALANINE

Sample No.	[A]/[I]	x	pH of suspension or solution[a)]	Mole of HCl required for dissolution 10^{-3} mol./g.
PADA(A431)	5	5.4	7.4	1.13
〃 (A432)	10	10.2	6.7	0.50
〃 (A433)	15	14.6	6.5	0.24
〃 (A434)	20	18.5	6.1	0
Poly-DL-alanine (J928)	50	28.8	3.7	0

a) 0.75 g. sample in 100 ml. water

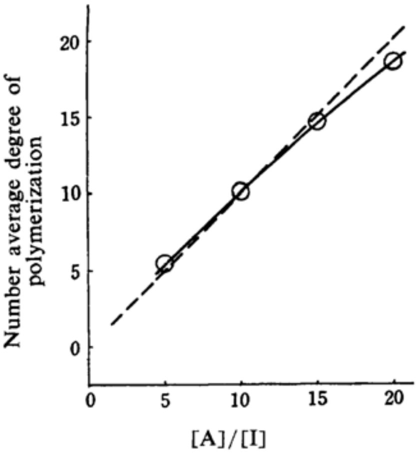
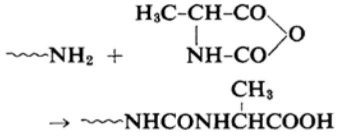


Fig. 1. PADA from polymerization in dioxane with dodecylamine initiator; number average degree of polymerization (x) as a function of anhydride-initiator ratio ([A]/[I]), (dashed line represents "theoretical" x-[A]/[I] relationship).

The number average degree of polymerization of PADA was determined by the titration of a terminal amino group with perchloric acid[7]. It should be equal to the [A]/[I] value, and Table I and Fig. 1 show that this relation is nearly fulfilled. Hereafter, the designation x is used instead of [A]/[I] for specifying a PADA preparation, although the value of x is not strictly equal to that of [A]/[I]. Values of x were slightly lower than the [A]/[I] value at higher [A]/[I] ratios. The discrepancy may be ascribed either to the error in the titration which is enhanced at higher [A]/[I] ratios or to the presence of a few thermal carboxyl groups which has been formed in a termination reaction during the polymerization in the following manner[7]:



The results of the terminal amino analysis and infrared data as described below confirm the formation of PADA, i. e., poly-DL-alanine linked with dodecylamine in its terminal carboxyl group, in the case of the polymerization of *N*-carboxy-DL-alanine anhydride initiated with dodecylamine.

PADA is insoluble in ordinary organic solvents such as acetic acid, chloroform, and dimethylformamide, but is soluble in formic acid, dichloroacetic acid and *m*-cresol.

PADA of $x > 17$ is soluble in water, but PADA of $x < 17$ gives a turbid solution in water, the turbidity increasing with decreasing x. Polyoxyethylene dodecylamine becomes soluble in water for the degree of polymerization higher than 8[8]. However, PADA of any x value is soluble in an aqueous acid solution, the free terminal amino group being ionized.

While the aqueous solutions of polyoxyethylene derivatives are known to exhibit the clouding phenomenon when heated[9], no such phenomenon is observed for PADA solution. This may be attributed to the formation of stronger hydrogen bonding between DL-alanyl residue and water molecule than that between oxyethylene residue and water molecule, the former being kept unbroken even at high temperatures.

Experimental

***N*-Carboxy-DL-alanine Anhydride.**— DL-Alanine (20 g.) was suspended in dry dioxane (300 ml.), stirred and phosgene bubbled in for about 3 hr., maintaining the temperature at 35~40°C. After the completion of the reaction carbon dioxide was passed through the solution until free of phosgene. The solvent was removed at reduced pressure. Then anhydrous ether was added and evaporated under reduced pressure, and this procedure was repeated several times. Dry petroleum ether was poured into an oily residue, and DL-alanine NCA was recrystallized after keeping the oil for 24 hr. at −15°C. DL-Alanine NCA was recrystallized from ether-petroleum ether. M. p. 45°C, yield 80%.

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[7] A. Berger and M. Sela, *J, Am. Chem. Soc.*, 77, 1893 (1955).

[8] T. Otsuka, personal communication.

[9] L. Raphael, *Ist. Int. Cong. on Surface Active Agent*, 1, 52 (1954).

Poly-DL-alanine Dodecylamide.—After an appropriate amount of dodecylamine (for example, 1.6 g. at [A]/[I]=10) was dissolved in dry dioxane (200 ml.), DL-alanine NCA (10 g.) was added to it at room temperature. Polymerization was allowed to proceed for several hours. The suspension of PADA was poured into a large amount of ether, the precipitate was gathered by centrifugation and dried in vacuo.

Other Derivatives of Poly-DL-alanine.—Poly-DL-alanine cetylamide and poly-DL-alanine *N*-carboxyethyl-myristylamide were synthesized in the same manner as described above, using cetylamine and *N*-myristyl-β-alanine as initiator, respectively. Poly-DL-alanine was prepared by polymerizing with triethylamine-initiation at [A]/[I]=50, the average x of which was found to be 28.8 by end-group analysis.

Titration of Terminal Amino Groups. — A N/20 perchloric acid solution was prepared by adding calculated amount of 70% perchloric acid to glacial acetic acid. After standing overnight, this solution was standardized by titration with pure sodium carbonate in glacial acetic acid, using crystal violet (0.2% glacial acetic acid solution) as indicator.

A sample of 50~80 mg. was weighed into a 50 ml. Erlenmeyer flask, dissolved in 2 ml. of dichloroacetic acid, and then 8 ml. of glacial acetic acid was added to the solution. The solution was titrated to green end-point of crystal violet indicator with the standard perchloric acid solution which was delivered from a 5 ml. micro-burette; the titer of solvent alone was subtracted from sample titer.

To check the procedure, a known amount of dodecylamine was titrated, and the agreement with calculated value was obtained at the green end-point. The point of color change was made somewhat obscure in the presence of dichloroacetic acid, but the accuracy of titration was within about 5%.

Infrared Measurements. — All infrared measurements were performed on a Shimadzu Type AR-275 double beam spectrometer using a sodium chloride prism. The samples for infrared measurements were prepared as dispersions either in potassium chloride disks or in Nujol.

Titration with an Aqueous Hydrochloric Acid Solution. — pH measurements were made with a Horiba Type M-3 pH-meter. A given amount of sample was dispersed or dissolved in distilled water, and titrated with a standard hydrochloric acid solution (0.06 N) to obtain a titration curve. Concentration of each sample was 0.75 g. polymer in 100 ml. solution.

Measurements of Surface Tension. — Surface tension was measured by drop weight method. The apparatus was placed in a thermostat of 25.0°C. The observed values were corrected by using the correction factor of Harkins and Jordan. The pH of sample solutions was adjusted to pH 5.0 by hydrochloric acid, unless otherwise stated, because all the samples were soluble at this pH.

Measurements of Surface Pressure. — Surface pressure was measured by a balance of hanging plate type at room temperature. Samples were spread from solutions in a mixed solvent, formic acid-isopropyl alcohol-water (1 : 2 : 7), on distilled water and on an aqueous solution of 0.01 M potassium carbonate or 0.2 M potassium chloride.

Results and Discussion

Infrared Absorption Spectra. — The infrared spectra of PADA and poly-DL-alanine were recorded in the solid state from potassium bromide disks and Nujol suspensions. Some of the infrared data are summarized in Table II.

The absorption bands at 2970 and 2930 cm^{-1} from a potassium bromide disk are assigned to the C–H stretching modes of CH_3 and CH_2 groups, respectively. The intensity of the former, relative to that of the latter, becomes stronger with increasing x, and is the strongest for poly-DL-alanine. This observation supports the formation of poly-DL-alanine linked with dodecylamine.

PADA and poly-DL-alanine show amide I absorption at 1661 and 1629 cm^{-1} and amide III absorption at 1240 and 1220 cm^{-1}. The lower frequency in the amide III band was observed in Nujol suspension but not in potassium bromide disk. A band due to N–H stretching lies at 3310 and 3280 cm^{-1}. The lower frequency appeared in potassium bromide disk for PADA of $x=5$ but not in Nujol suspension. In amide I band the higher frequency

TABLE II. CHARACTERISTIC ABSORPTIONS OF PADA AND POLY-DL-ALANINE

Characteristic band		Frequency, cm^{-1}				
		PADA (x=5)	PADA (x=10)	PADA (x=15)	PADA (x=20)	Poly-DL-alanine
N-H(str.)[a]			—	3310 (s)	—	3310 (s)
		3280 (s)	—		—	
Amide I		1661 (sh)	1661 (sh)	1661 (s)	1661 (s)	1661 (s)
		1629 (s)	1629 (s)	1629 (s)	1629 (s)	1629 (s)
Amide II		1530 (s)	1530 (s)	1530 (s)	1530 (s)	1530 (s)
Amide III[b]		1240 (w)	1240 (w)	1240 (m)	1240 (m)	1240 (m)
		1220 (m)	1220 (m)	1220 (m)	1220 (w)	1220 (sh)
C-H(str.)[a]	CH_3-	2970 (w)	—	2970 (w)	—	2970 (m)
	CH_2-	2930 (s)	—	2930 (m)	—	2930 (w)

str.=stretching mode, s=strong, m=medium, w=weak, sh=shoulder

a) from KBr disk, b) from Nujol suspension of the two amide bands is characteristic of the random conformation of polypeptide and the lower is of the β-conformation[10-13],*. The dependence of the relative intensity of two frequencies in the amide III and the N–H stretching bands upon the value of x is also related to the presence of these two conformations. In all the three bands the intensity of the higher frequency of PADA relative to that of the lower increases with x, and poly-DL-alanine absorbs the higher frequency strongly. The results indicate that the random conformation is predominant for PADA with higher x ($x \geq 20$) and poly-DL-alanine, the portion of the β-conformation increases with lowering x, and PADA with $x=5$ is mostly in the β-conformation**.

However, the above correlation of infrared frequency with the conformation of polypeptides could not be found in amide II band.

Titration with an Aqueous Hydrochloric Acid Solution. — When PADA or poly-DL-alanine is dispersed or dissolved in water, the solution shows an acidic pH. Poly-DL-alanine gives the most acidic pH, 3.7. Table I shows the pH's of the solution of 0.75 g. polymer in 100 ml. A value of pH, 2.8, of poly-DL-alanine ($x=30$) solution has been reported already[15]. This might be ascribed partly to the presence of the terminal carboxyl groups produced by the termination reaction during polymerization, as mentioned before[7].

The solution of PADA exhibits lower pH with increasing x, because an equal gram of polymer contains less terminal amino groups with increasing x. This effect is enhanced by the more complete solubility of PADA with higher x, since the amino groups of insoluble molecules would remain unionized.

When the solution of PADA is titrated with an aqueous hydrochloric acid solution, the pH is gradually lowered and then suddenly decreases. Fig. 2 shows the pH of solution plotted against mole of hydrochloric acid added when 0.75 g. PADA is dispersed or dissolved in 100 ml. solution. On this titration curve, a break point exists at about pH 5.5, irrespective of x. At this point the turbid dispersion of PADA with $x<17$ becomes almost transparent. If all the terminal amino groups could be titrated with hydrochloric acid, the break point should be the end point, and the mole of hydrochloric acid consumed should be equal to mole of terminal amino groups obtained from anhydrous perchloric acid titration. Nevertheless, the former was found to be considerably smaller than the latter. The discrepancy might arise from the fact that the PADA molecules in aqueous acid media aggregate into a "micelle". The micelle is composed of ionized molecules together with unionized molecules, the latter being not titratable with hydrogen ions. Evidence for the micelle formation is provided by surface tension data and preliminary experiments of ultracentrifugation and solubilization.

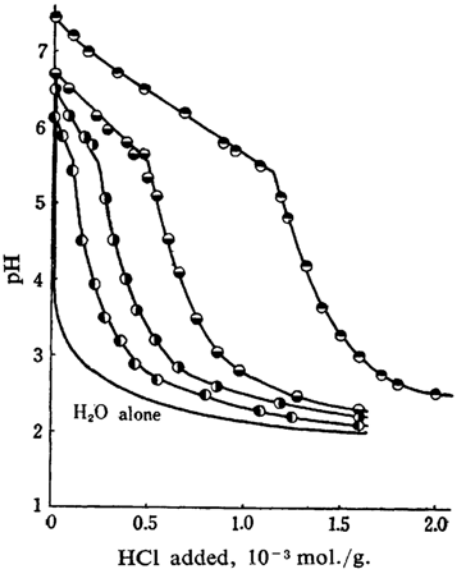

Fig. 2. Plots of pH of PADA solution against added amout of hydrochloric acid at 25°C. ◓, $x=5$; ◒, $x=10$; ◑, $x=15$; ◐, $x=20$

Surface Tension. — The surface tension of aqueous solutions of PADA together with poly-DL-alanine and dodecylamine hydrochloride was measured at pH 5.0. Dodecylamine hydrochloride was a sample recrystallized several times from acetone-benzene. The surface tension vs. concentration curves at pH 5.0 are shown in Fig. 3. The result obtained for dodecylamine hydrochloride was in good agreement with that already reported[16].

The surface tension of PADA solution is

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[10] A. Elliott, *Nature*, **170**, 1066 (1952).

[11] C. H. Bamford, A. Elliott and W. E. Hanby, "Synthetic Polypeptides", Academic Press, New York (1956), p. 153.

[12] A. Elliott, *Proc. Roy. Soc.*, **A221**, 104 (1953).

[13] T. Miyazawa and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 712 (1961).

* Following Elliott and others[10-12], the absorption at 1661 cm^{-1} arises from the α-form.

** It is reported[14] that a low molecular weight poly-γ-benzyl-L-glutamate differs from a high molecular weight polymer in its conformation, while the latter is in the α-helical conformation.

[14] E. R. Blout and A. Asadourian, *J. Am. Chem. Soc.*, **78**, 955 (1956).

[15] K. Linderstøm-Lang, "Symposium on Protein Structure", Ed. by A. Neuberger, Methuen and Co. Ltd., London (1958), p. 23.

[16] E. J. Hoffman, G. E. Boyd and A. W. Ralston, *J. Am. Chem. Soc.*, **64**, 408 (1942).

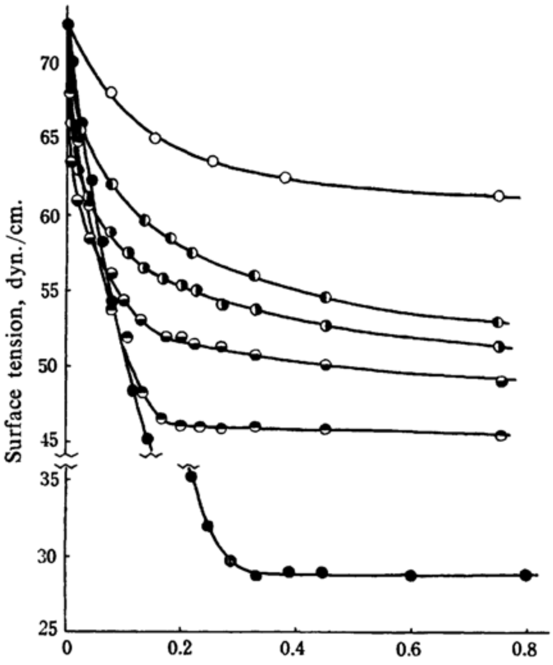

Fig. 3. Surface tension vs. concentration curves for aqueous solution of PADA, poly-DL-alanine and dodecylamine hydrochloride at pH 5.0 25°C. PADA: ◓, $x=5$; ◒, $x=10$; ◑, $x=15$; ◐, $x=20$; ○, poly-DL-alanine; ●, dodecylamine hydrochloride

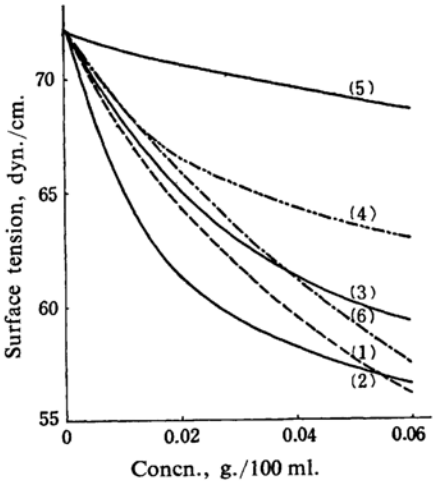

Fig. 4. Surface tension vs. concentration curves of PADA, poly-DL-alanine and dodecylamine hydrochloride at dilute concentrations. pH 5.0, 25°C. PADA: (1) $x=5$, (2) $x=10$, (3) $x=15$, (4) $x=20$, (5) poly-DL-alanine, (6) dodecylamine hydrochloride

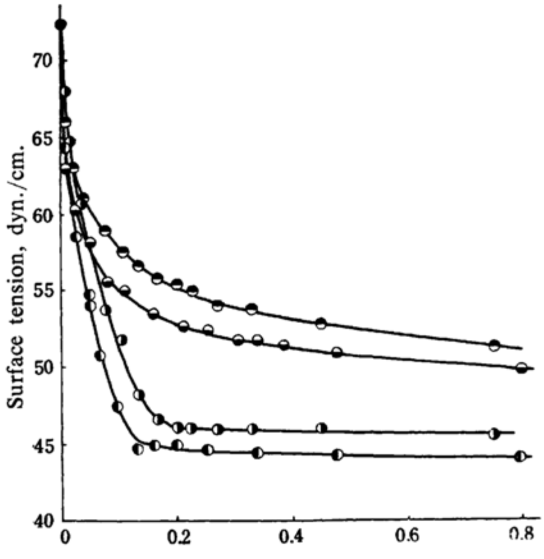

Fig. 5. Effect of acid on surface tension of PADA at 25°C. $x=5$: ◑, pH 5.0; ◐, pH 2.2, $x=15$: ◓, pH 5.0; ◒, pH 2.2

characterized by a sharp initial decrease followed by a gradual flattening, as the concentration becomes higher. This behavior is commonly observed for surface active agents. The break point corresponds to the critical micelle concentration, although it becomes obscure for PADA of higher x. The critical micelle concentration of PADA lies in more dilute concentration than that of dodecylamine hydrochloride, and shifts towards lower concentration as x increases.

In a PADA molecule, a sequence of DL-alanyl residues is between the dodecyl group and the ionized or unionized hydrophilic amino group at pH 5.0, where PADA is partially ionized. In this case the part of poly-DL-alanine reduces the repulsion between ionized hydrophilic groups due to the intermolecular hydrogen bonding. Thus the micelle formation takes place readily at lower concentration as x increases. It is noticeable that the critical micelle concentration of PADA lies at about 10^{-3} mole per liter which is compared with that of polyoxyethylene derivatives with long alkyl chain, 10^{-4} mol. per liter[17].

At low concentrations the slope of surface tension vs. concentration curve is the steepest for PADA of $x=10$ and is more gentle when x is either smaller or larger than this value, as shown in Fig. 4. For $x<10$, the explanation is as described before. For $x>10$ the attraction

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[17] L. Hsioa, H. M. Dunning, P. B. Lorenz, *J. Phys. Chem.*, **60**, 657 (1956). E. Gonick and J. W. McBain, *J. Am. Chem. Soc.*, **69**, 334 (1947).

due to hydrogen bonding between DL-alanyl residues predominates over van der Waals cohesion between alkyl chains and the characteristics of poly-DL-alanine become obvious and reduce the effect of alkyl chain, thus surface tension being less lowered.

Fig. 5 show the effect of acid on surface tension of PADA. The more acidic the solution of PADA is, the lower the surface tension becomes and the break point shift to more dilute concentration. This fact indicates that the excess acid added beyond pH 5.5 remains free in solution and behaves like a simple neutral salt, suppressing the electrostatic repulsion between ionized amino groups.

Surface Film of PADA. — Fig. 6 illustrates surface pressure vs. area curves of PADA and poly-DL-alanine films on distilled water. They are of condensed type. The limiting area, $A_{\Pi\to0}$ (m²/mg.), of PADA increases with x, tending to that of poly-DL-alanine, as listed in Table III. The film of PADA occupies a larger area on the aqueous subphase containing salt than on distilled water, as shown in Fig. 7, but salt has no influence on poly-DL-alanine film.

The limiting area expressed in m²/mg. is reduced to that in Å² per average DL-alanyl residue by

$$A_{\Pi\to0}(\text{Å}^2/\text{residue}) = \frac{10^{23}}{N}\left(M_{\text{A}}+\frac{M_{\text{B}}}{x}\right)A_{\Pi\to0}\ (\text{m}^2/\text{mg.}) \qquad (1)$$

if the residue weight of dodecylamine is equally distributed over alanyl residues. Here N is the Avogadro number, and M_{A} and M_{B} are residue weight of alanine (71) and dodecylamine (184), respectively. In Eq. 1 x is assumed to be infinite for poly-DL-alanine film.

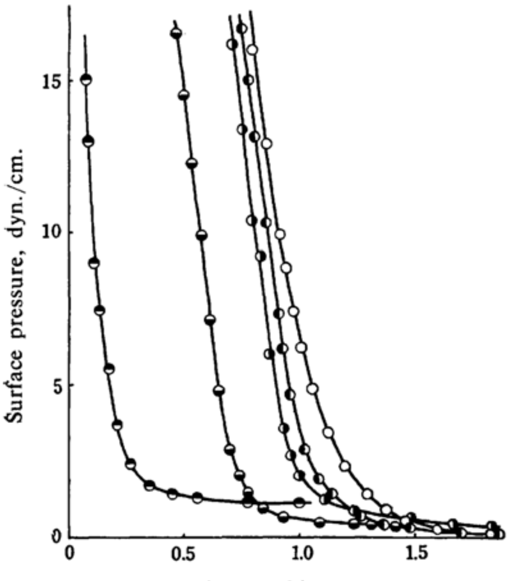

Fig. 6. Surface pressure vs. area curves of PADA and poly-DL-alanine on distilled water at 18°C. PADA: ◓, $x=5$; ◒, $x=10$; ◑, $x=15$; ◐, $x=20$; ○, poly-DL-alanine

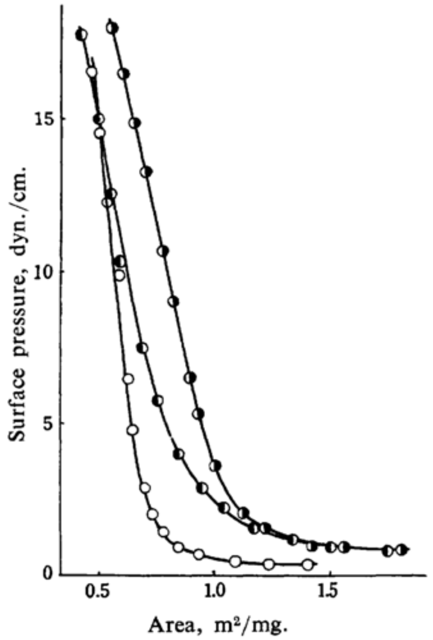

Fig. 7. Effect of salts on the surface pressure vs. area curves of PADA with $x=10$ at 18°C. ○, no salt; ◐, 0.01 M potassium carbonate; ◑, 0.2 M potassium chloride

TABLE III. LIMITING AREAS AND f VALUES OF PADA AND POLY-DL-ALANINE

x	On distilled water $A_{\Pi\to0}$			On 0.01 M K_2CO_3 $A_{\Pi\to0}$			On 0.2 M KCl $A_{\Pi\to0}$		
	m²/mg.	Å²/residue	f	m²/mg.	Å²/residue	f	m²/mg.	Å²/residue	f
5	0.26	4.7	0.05	—	—	—	—	—	—
10	0.74	11.0	0.64	0.89	13.3	0.81	1.07	15.9	0.99
15	0.98	13.5	0.87	1.09	15.0	0.98	1.11	15.3	1.00
20	1.06	14.1	0.94	—	—	—	—	—	—
Poly-DL-alanine	1.18	14.0	—	—	—	—	1.18	14.0	—

Since the limiting area of long chain alkylamine or alkylamide is about 20 Å^2 per molecule[18] and that of poly-DL-alanine is 14 Å^2 per residue, PADA should have a limiting area

$$A_{\Pi\to0}(\text{Å}^2/\text{residue})=\frac{20+14x}{x} \qquad (2)$$

if it spreads completely as a monomolecular film. While the observed value of the limiting area of PADA on aqueous salt solution such as 0.01 M potassium carbonate or 0.2 M potassium chloride is found to agree with the value calculated from Eq. 2, that obtained on distilled water is smaller than the calculated value. In the latter case, a part of hydrophilic poly-DL-alanine part might be submerged into water. If it is assumed that xf DL-alanyl residues and a dodecylamino residue in a PADA molecule form a surface film and $x(1-f)$ DL-alanyl residues are submerged into water the limiting area of PADA may be expressed by

$$A_{\Pi\to0}(\text{Å}^2/\text{residue})=\frac{20+14xf}{x} \qquad (3)$$

Substituting the observed values of limiting area for $A_{\Pi\to0}$(Å^2/residue) in Eq. 3, the values of f are obtained as given in Table III.

It is interesting to note that, while PADA becomes more hydrophilic and more soluble in water as x is larger, in surface film it spreads more; that is, more DL-alanyl residues in a molecule exist on an aqueous surface. This observation may be interpreted as follows: When PADA of lower x is compressed, the van der Waals attraction between dodecyl groups is so strong that some of DL-alanyl residues are forced to be submerged into water. For PADA of higher x, however, a hydrogen-bonded conformation[19-21] of poly-DL-alanine part will remain unbroken on compression, since the hydrogen bonding is more predominant than van der Waals cohesion.

Summary

Poly-DL-alanine alkyl amide has been synthesized by the polymerization of *N*-carboxy-DL-alanine anhydride initiated with alkyl amine at different anhydride-initiator ratios ([A]/[I]). The amphipathic properties of poly-DL-alanine dodecylamide (PADA) with [A]/[I] = 5 to 20 have been investigated in comparison with poly-DL-alanine.

The degree of polymerization of poly-DL-alanine part can be easily controlled by the initial [A]/[I] ratio. The number average degree of polymerization (x) is found to be approximately equal to [A]/[I].

The solubility of PADA in water is higher as x increases, and PADA with $x>17$ is transparently soluble. PADA with any x value is soluble in aqueous acid solutions, the pH of which is lower than 5.5. The titration curve with an aqueous acid solution shows a break at this pH.

The infrared spectra of PADA in the solid state indicate that PADA with higher x is almost in the random conformation and the portion of the β-conformation increases as x becomes lower.

Surface tension of PADA in aqueous acid solution exhibits typical characteristics of amphipathic substances. The degree of surface tension lowering at low concentration region is the largest for PADA of $x=10$.

The surface film of PADA is of condensed type and the limiting area is larger as x increases. PADA with higher x becomes more spread as surface film, more DL-alanyl residues being on the aqueous surface.

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