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Monolayers of Poly-γ-benzyl-L-glutamate *N*-Alkylamides

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To elucidate the effect of long alkyl chains on the surface properties of polypeptides, various poly-γ-benzyl-L-glutamate (PBLG) hexylamides or dodecylamides with different degrees of polymerization were synthesized and their monolayers were examined. PBLG-alkylamides with a low degree of polymerization form expanded-type monolayers on water and their surface pressure-area (π-A) curves have two plateaus. The lower plateau in the π-A curve is not seen in the curves of PBLG-alkylamides with a degree of polymerization of more than 10. The lower plateau in the π-A curve is due to the behavior of the terminal alkyl groups. The effect of temperature on the properties of monolayers was examined. Structures formed in the monolayers were discussed.

From studies on the relation between surface pressure and surface viscosity, it was concluded[1] that the monolayer of poly-γ-benzyl-L-glutamate (PBLG) of low molecular weight is of an expanded type. This was done by comparing polypeptides of rather high molecular weight. The work dealt with the properties of monolayers of low molecular weight PBLG which has a long alkyl chain at its terminal. Ikeda *et al.*[2] discussed to what extent hydrogen bonds between peptides and the van der Waals force between alkyl chains of long chain alkyl amides of poly-α-amino acids contribute to micelle formation in water. It is of interest to see how the van der Waals force between alkyl chains and hydrogen bonds between peptides act in monolayers of polypeptide derivatives, because PBLG-alkylamides are water-insoluble and form monolayers on water.

For this purpose, *n*-hexyl and *n*-dodecyl groups were introduced into polypeptides with different degrees of polymerization. Monolayers of these polymers seemed suitable for studies on the factors making a monolayer stable and on the structures formed in monolayers.

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[1] A. Suzuki and T. Isemura, *Ann. Rep. Biol. Works, Osaka Univ.*, **15**, 75 (1967).

[2] S. Ikeda, M. Maekawa, and T. Isemura, This Bulletin., **39**, 105 (1966).

Experimental

A long chain alkylamide of poly-γ-benzyl-L-glutamate was prepared by polymerization of *N*-carboxy-γ-benzyl-L-glutamate anhydride, mp 93°C, in dioxane using hexylamine or dodecylamine as an initiator.[3] The molar ratio of anhydride to initiator, [A]/[I], was varied from 5 to 25 to obtain samples of PBLG-dodecylamide with different degrees of polymerization. The [A]/[I] ratios of the PBLG-hexylamide series were 7, 14, 21, and 28.

The degree of polymerization of polymers was estimated by anhydrous titration of terminal amino groups.[4] Polymers were dissolved in acetic acid and titrated with perchloric acid using methylviolet as an indicator. The activity of perchloric acid was determined by titration with γ-benzyl-L-glutamic acid. The average degrees of polymerization estimated by the titration of amino end groups were approximately equal to the molar ratios of anhydride to initiator: all the ratios of the average degree of polymerization to the [A]/[I] ratio were between 0.9 and 1.2. The polymers were not fractionated into a monodisperse system, so the degrees of polymerization were taken as the [A]/[I] values. In this paper, the [A]/[I] value is given as a suffix *e.g.* PBLG-hexylamide-7 has a value of 7.

Dichloroacetic acid was distilled twice under reduced pressure before use. Benzene was distilled three times. Potassium chloride was heated for about 3 hr at its partial melting temperature.

Polymers were spread at an air/water interface using a conventional Langmuir trough (50.0×14.5×0.9 cm) and their surface properties were measured by means of automatic recording systems.[5] The hanging plate method was employed for measuring surface pressure, the rotatory surface viscometer for surface viscosity and the vibrating electrode method for surface potential.

Polymers were dissolved in a mixture of dichloroacetic acid and benzene (1 : 4, by volume) and spread on water using a micrometer syringe (Alfa, Takara). Potassium chloride was added to the aqueous phase at a concentration of 1 mM to stabilize surface potential. Temperature was controlled by circulating water at constant temperature through a coiled glass tube at the bottom of the trough.

Results and Discussion

The surface pressure (π) and surface viscosity (η) *vs.* surface area (A) curves for the series of PBLG-hexylamide at 20°C are shown in Fig. 1. PBLG-hexylamide-7 has an unusual π-A curve which is of a very expanded type and has two plateaus at surface pressures of 7.8 and 10.0 dyn/cm. The π-A curves of PBLG-hexylamides other than PBLG-hexyl-7 became more condensed as the degree of polymerization increased and their π-A curves had a single plateau at an area of 0.5 m^2/mg. Surface viscosity could be detected in monolayers of polymers with a high degree of polymerization when the area was large. Areas where surface viscosities were first detected were distrubuted in the region of the plateaus of the π-A curves.

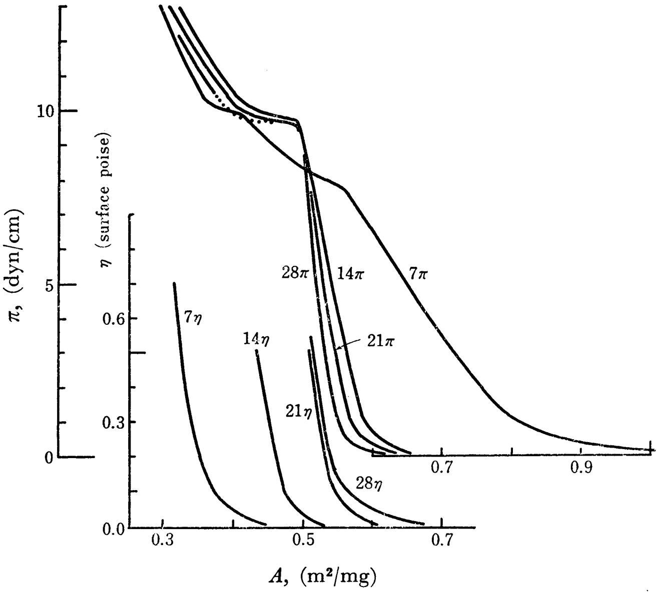

Fig. 1. Variation of the surface properties of PBLG-hexylamide series on water at 20°C.
The degree of polymerization is noted on each curve.

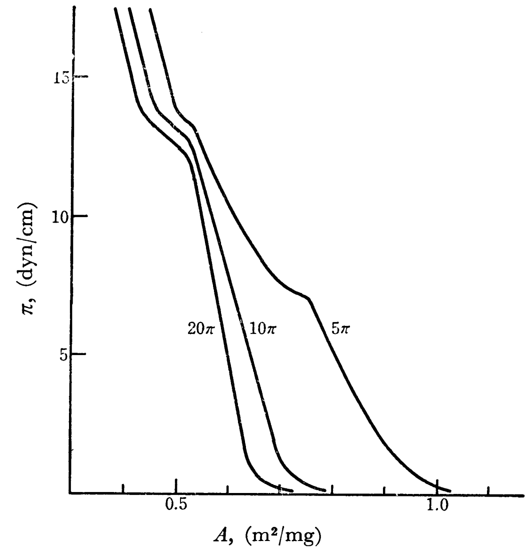

Fig. 2. Variation of π-A curves of PBLG-dodecylamide series on water at 20°C.
The degree of polymerization is noted on each curve.

The π-A and η-A curves of the PBLG-dodecylamide series at 20°C are shown in Fig. 2. There are two plateaus at π=7.2 and 13.0 dyn/cm in the π-A curve of PBLG-dodecylamide-5. The π-A curves of the PBLG-dodecylamide series become a more condensed type with increase in the degree of polymerization. The π-A curves of this series have higher plateaus in the area of 0.55 m^2/mg. The surface pressure corresponding to the beginning of the higher plateau varied with the degree of polymerization, but those of the end of the plateau were all at the same pressure of 14.6 dyn/cm.

The temperature dependence of the π-A curve of PBLG-hexylamide-7 is given in Fig. 3. The surface pressure of the lower plateau of the π-A curve does not change appreciably with temperatue but the

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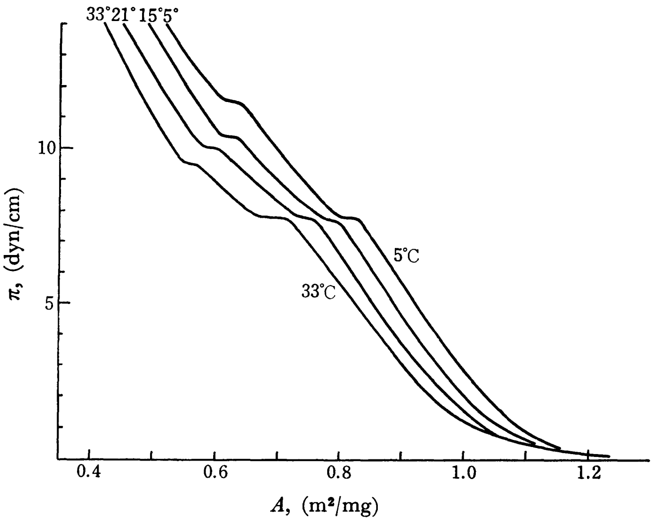
[3] E. R. Blout and R. H. Karlson, *J. Amer. Chem. Soc.*, **78**, 941 (1956).

[4] M. Sela and A. Berger, *ibid.*, **77**, 1893 (1955).

[5] A. Suzuki, S. Ikeda, and T. Isemura, *Ann. Rep. Biol. Works, Osaka Univ.*, **15**, 83 (1967).



T °C	π_{p1}	π_{p2}
5	7.8	11.5
15	7.8	10.6
21	7.8	10.0
33	7.8	9.5

Fig. 3. Temperature dependence of the π-A curves for PBLG-hexylamide-7 on water.
The critical surface pressure π_p related to the plateaus are shown in the figure.

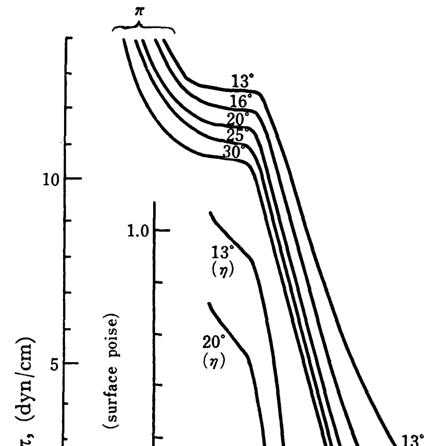


Fig. 4. Temperature dependence of the π-A and η-A curves for PBLG-dodecylamide-10 on water.

surface area corresponding to the beginning of the plateau became smaller with increasing temperature. Both the surface pressure and area of the higher plateau varied with temperature. Temperature had similar effects on the π-A curve of PBLG-dodecylamide-5. Variation of π-A curves of PBLG-dodecylamide-10 with temperature is shown in Fig. 4. The surface pressure corresponding to the higher plateau decreases at high temperature.

The surface moment *vs.* area curves of the PBLG-dodecylamide series are shown in Fig. 5. The surface moment was calculated by the Helmholtz equation $\mu = \Delta VA/4\pi$, assuming that the area occupied by a terminal alkylamine residue is similar to that occupied by an amino acid residue. Since the limiting area of an amino acid residue has been found to be 22 Å^2 in a monolayer of PBLG[1,6] and that of dodecylamine 20.5 Å^2,[7] the above assumption is probably true for a closely packed film.

The smallest area showing the maximum surface moment corresponds to the lower plateau of the π-A curve with PBLG-dodecylamide-5 and also to the area where the surface pressure becomes detectable with PBLG-dodecylamide-10 and PBLG-dodecylamide-20. In an area smaller than that of the plateau, the μ-A curves decrease linearly. In the region from the smallest area showing the maximum surface moment to the area where the surface moment begins to decrease linearly, the plot of μ against A is curved. Assuming that molecules in a monolayer remain in the same conformation when the surface moment is constant, the conformations of the polypeptides seem to remain the same until the surface pressure becomes about 1 dyn/cm, except for PBLG-dodecylamide-5 and PBLG-hexylamide-7 which remain in the same conformations until the lower plateaus are reached. In the region where the plot is curved, the terminal groups contribute considerably to the surface moment since the surface moment varies more for polypeptides of low molecular weight. This suggests that reorientation of terminal groups of polypeptides in associated with decrease in the surface area in this region.

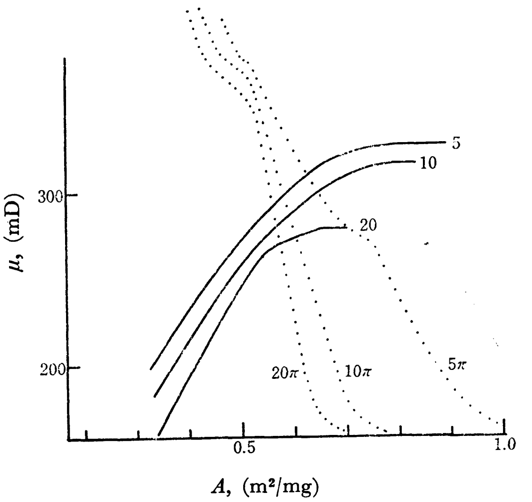


Fig. 5. Surface moment *vs.* area curves for PBLG-dodecylamide series.
The degree of polymerization is noted on each curve.
The π-A curves are shown by dotted lines.

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[6] S. Ikeda and T. Isemura, This Bulletin,. **34**, 416 (1961).

[7] N. K. Adam, *Proc. Roy. Soc.* (London), **A103**, 687 (1923).

Two components of surface moment, due to a peptide and to a terminal group, were estimated from the maximum surface moment, as μ(peptide)$=266\pm29$ and μ(terminal)$=450-1100$ mD. The μ value (peptide) is in accord with that of 265 mD obtained for a monolayer of PBLG.[1,6] The μ value(terminal) varied greatly with polymers. This might be due to complicated factors, such as ionization of these groups.

A hydrocarbon chain should influence the properties of monolayers of polymers of low molecular weight, so the π-A curves of PBLG-hexylamide-7 and PBLG-dodecylamide-5 were compared. The π-A curve of PBLG-hexylamide-7 was found to be more expanded than that of PBLG-dodecylamide-5. The π-A curves of monolayers of PBLG reported previously[1,6] had only one plateau corresponding to the higher plateau observed in this work. This suggests that the lower plateau is due to the terminal alkyl chains. This is supported by the finding that the lower plateau disappeared as the ratio of alkyl groups to peptides became smaller with increase in the degree of polymerization. It seems likely that as the interaction between hexyl groups is smaller than that of dodecyl groups the monolayer of PBLG-hexylamide-7 is more expanded than that of PBLG-dodecylamide-5. The surface pressure related to the lower plateau, which is independent of the temperature, is 7.8 dyn/cm for PBLG-hexylamide-7 and 7.2 dyn/cm for PBLG-dodecylamide-5. Langmuir[8] formulated the interaction between hydrocarbon chains as a function of the number of carbon atoms. Since the polypeptides used in this work have hydrocarbon chains in both their terminals and their side chains, quantitative analyses of their interaction are difficult. The surface pressure at the higher plateau was 10 dyn/cm for PBLG-hexylamide-7 at 20°C and 13 dyn/cm for PBLG-dodecylamide-5 at 20°C, and decreased with increasing temperature.

Surface viscosities become detectable at areas corresponding to the higher plateaus in the π-A curves. It has been reported[9,10] that the surface viscosity of polypeptides is due to the formation of hydrogen bonds between polypeptides. This suggests that hydrogen bonds are formed between peptides in the monolayers when the area corresponds to the higher plateau.

From the shape of the π-A curves obtained with this series of polypeptides, it seems likely that the changes occurring in the monolayers are as follows: (1) molecules in the monolayers become aggregated by the van der Waals force between hydrocarbon chains, (2) reorientation of hydrocarbon chains occurs in the region of the lower plateau, (3) molecules combine by formation of hydrogen bonds between peptide parts of the molecule and (4) reorientation of the side chains of amino acid residues occurs in the region of the higher plateau. The polymers may come in contact with each other by interaction of hydrocarbon chains and the interaction might be too weak to cause any detectable surface viscosity at an area larger than that of the lower plateau. The surface properties of monolayers of PBLG were explained by Yamashita and Yamashita[11] in relation to conformation of the polypeptide. If the conformational change of the oligopeptide studied in this work is considered, it may be related to the higher plateau. Examination of the infrared spectra of the PBLG-dodecylamide series in solid film indicated that low molecular weight PBLG-dodecylamide in the solid film predominantly had β-structure.[12] The conformation of polypeptides in solid films might be related to the π-A curves, as suggested by Yamashita and Yamashita. However, at present information available for further discussion is still insufficient.

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[8] I. Langmuir, *J. Chem. Phys.*, **1**, 754 (1933).

[9] S. Ikeda and T. Isemura, This Bulletin., **32**, 659 (1959).

[10] N. L. Gershfeld and C. Y. C. Pak, *J. Colloid Sci.*, **23**, 215 (1967).

[11] T. Yamashita and S. Yamashita, This Bulletin, **43**, 3969 (1970).

[12] Solid films for the examination of infrared spectra were prepared from solutions in chloroform on silver chloride plates. Infrared spectra were recorded with a Perkin-Elmer Spectrophotometer, model 221, using a sodium chloride prism. The existence of the α-helical or β-structure is deduced from the absorption bands of amide I and II of polypeptides.