Surface Chemistry of Synthetic Protein Analogues. II. Temperature Dependence of the Force-Area Relations of Some Polypeptides Monolayers and Thickness of the Monolayer*

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Introduction

Previously, the authors studied the various kinds of the behavior of the monolayers of some synthetic polypeptides as the model of natural proteins.[1] The polypeptides were of non-ionic side chains from four to ten carbon atoms in length. The force-area relations of the films were first investigated. From the results obtained, it was concluded that poly-DL-α-aminocaproic acid resembles exceedingly natural proteins among others. It has the side chains of four carbon atoms and its limiting area occupied by the film at zero compression was ca. 15 Å^2 per amino-acid residue. On the other hand, it was found that a plateau which is a part of high compressibility appears in the force-area (F-A) curves of the monolayers of polypeptides of longer side chains such as poly-DL-α-aminocaprylic acid, poly-DL-α-aminocapric acid, poly-DL-α-aminolauric acid and poly-γ-benzyl-DL glutamate. The F-A curves of these polypeptides monolayers were quite different in shape from those of natural proteins. The end of the plateau was always about 15 Å^2 per residue with all these films mentioned above. This area corresponds to the area per residue calculated from X-ray data of close packed chains of polypeptide of β-keratine type. (when the side chains were compressed away from the surface and the backbones were closely packed.). The resemblance between F-A curves of the protein monolayer and that of synthetic polypeptide of short side chains such as poly-DL-α-aminocaproic acid is due principally to the fact that natural proteins have on the average the side chains of effective length of three carbon atoms or less.

In the previous paper, the appearance of the plateau in the F-A curve was attributed to the reorientation of the polymer molecule caused by the compression of monolayer. To prove our supposition, the energy required for reorientation was evaluated and the thickness of the monolayer before and after transition was determined. In the course of experiments, it was found that the plateau of the F-A curves moves to the lower pressure region with the rise of temperature. From the relation of temperature and the pressure of transition from one state of the film to the other reoriented state, the heat of transition was estimated by the aid of two-dimensional equation of Clapeyron-Clausius.

On the other hand, these polypeptides films were transferred on the optical guage made of the built-up film of barium stearate under the spreading pressures under and above the transition pressure using the various piston oils. Then, the thickness of the built-up film of these polypeptides films was measured optically.

From the experimental results, our views of the reorientation at the plateau in F-A curve were justified for the most part.

Experimental Methods

Surface pressure was measured by the hanging plate method as reported in the previous paper. Distilled water was used as substrate, for the monolayer of these polypeptides is scarcely affected by the acidity or basicity of the substrate, as reported in the previous paper. To regulate the temperature of the substrate in the trough, the glass hose was dipped in the trough, through which water at constant temperature was circulated from a thermostat.

The films of these polypeptides were built up on the optical guage made of barium stearate multilayers by Langmuir-Blodgett's technique[2,3]

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[1] T. Isemura and K. Hamaguchi, This Bulletin, **25**, 40 (1952). T. Isemura, K. Hamaguchi, H. Tani. J. Noguchi and H. Yuki, *Nature*, **168**, 165 (1951).

under various spreading pressures of piston oils such as castor oil, tricresyl phosphate and some piston oils of low spreading pressure. Piston oils of low spreading pressure were prepared by mixing ethyl myristate with liquid paraffin[4] and their spreading pressure was measured by film balance beforehand for use. The thickness of the films of these polymers was determined optically by Blodgett's method.[3]

Poly-DL-α-aminocaproic acid film could not be transferred on the optical guage by dipping the plate vertically into the water covered with monolayer. However, it was transferred if the plate was inserted into the aqueous phase at an angle of 15° with the water surface and then withdrawn holding the plate at the same inclination. Polypeptide films other than that of poly-DL-α-aminocaproic acid were built up in the usual manner.

Results

The observed force-area relations at various temperatures are shown in Figs. 1–4.

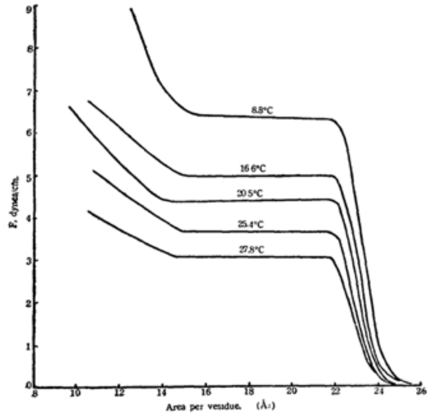

Fig. 1.—Poly-DL-α-aminolauric acid

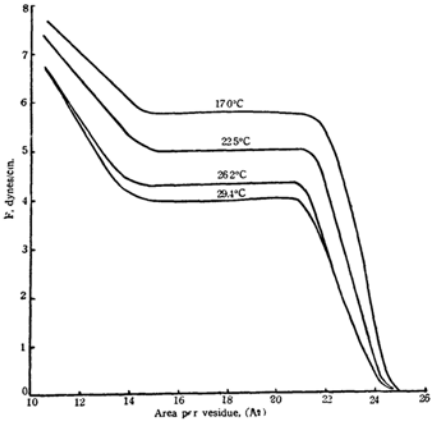

Fig. 2.—Poly-DL-α-aminocapric acid

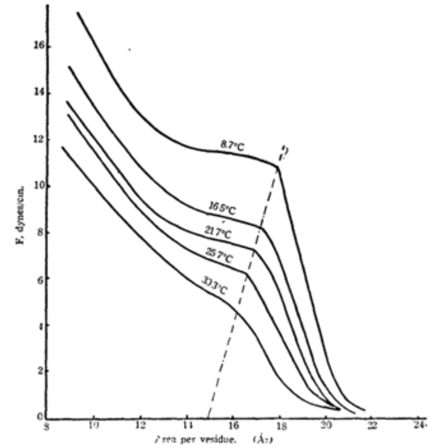

Fig. 3.—Poly-DL-α.aminocaprylic acid

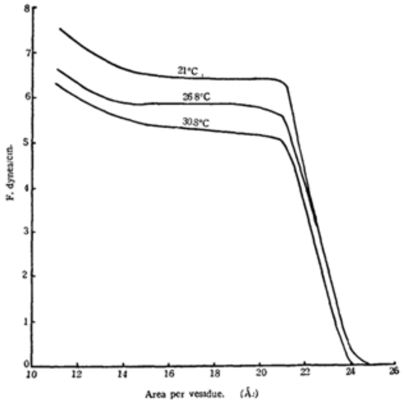

Fig. 4.—Poly-γ-benzyl-DL-glutamate

The force-area relation of the polymer film of short side chains such as poly-DL-norleucin was hardly influenced by the change of temperature. However, the force-area curves of the surface films of the polymers with longer side chains, such as poly-DL-α-aminolauric acid, poly-DL-α-aminocapric acid, poly-DL-α-aminocaprylic acid and poly-γ-benzyl-DL-glutamate were profoundly affected by the change of temperature. The plateau which appears in the F-A curves of monolayers of these polymers, passes to the lower pressure region with the rise of temperature of the substrate water. Nevertheless, the area at which the plateau begins to appear and the area at the end of the plateau, where the surface pressure begins to increase again were always nearly the same and independent of temperature. Moreover, the area of the end of the plateau was constant, namely, 15 Å² irrespective of the kind of polypeptides. The behavior of the monolayer of poly-DL-α-aminocaprylic acid was somewhat different from that of three other polypeptides. Although the plateau passes to the lower pressure region with the rise of temperature, the plateaus are not parallel to the area axis. In this case, their inclinations increase gradually and the area at which the plateau begins to appear, shifts to a

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[2] K. B. Blodgett, *J. Am. Chem. Soc.*, **57**, 1107 (1935).

[3] K. B. Blodgett and I. Langmuir, *Phys. Rev.*, **53**, 909 (1938).

[4] Norris and Taylor, *J. Chem. Soc.*, **1938**, 1719.

smaller area with the rise of temperature. The F-A curves of the monolayer of poly-DL-α-aminocaprylic acid may be an intermediate case between monolayers of polypeptide of shorter side chains and those of longer side chains.

The thickness of the built-up films of these polypeptides are shown in Table 1. The observed thickness h_3 indicated in the Table is not the value actually observed but the thickness which the built-up film should have, if the deposition ratio were unity. For comparison, the calculated thickness is also shown in the Table. h_1 is the thickness obtained by dividing the density of the polypeptide with the area per amino-acid residue at the spreading pressure of the piston oil used, which can be estimated from F-A curve. h_2 is the thickness of the film estimated by using the molecular model by Stuart, when the polypeptide chain is of β-keratine type and is compressed closely so as to project the side chains of the one side of the backbone and to push the side chains of the other side into the water.

Table 1.

	Spreading Pressure	Thickness (Å)		
		calculated		observed
Polypeptides	F (dynes/cm.)	h_1	h_2	h_3
Poly-DL-α-aminocaproic acid	3.1	10.7	11.7	11.1
Poly-DL-α-aminocaprylic acid	5.8	10.1	—	9.0
	9.5	15.0	16.4	15.6
Poly-DL-α-aminocapric acid	3.1	9.6	—	9.4
	9.5	24.0	21.3	22.7
Poly-DL-α-aminolauric acid	3.1	9.6	—	9.5
	5.8	23.9	26	20.7

Discussion

In the previous paper, the appearance of the plateau in the F-A curve was attributed to the reorientation of the polymer molecule caused by compression of monolayer. If we assume that both films before and after reorientation are two different surface phases, the surface pressure at which the plateau appears in the F-A curve, is the pressure of a phase transition. If this transition pressure was plotted against the temperature, straight lines were obtained for every polypeptide as shown in Fig. 5. dF/dT was readily evaluated from this figure. Inserting this value into two-dimensional Clapeyron-Clausius' equation, the change of enthalpy by the phase transformation of the film at any temperature can be evaluated as follows:

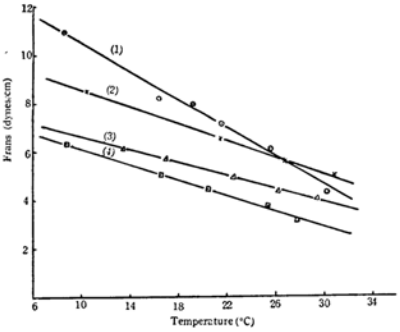

Fig. 5.—Transition pressure-temperature relation (1) Poly-DL-α-aminocaprylic acid; (2) poly-γ-benzyl-DL-glutamate; (3) Poly-DL-α-aminocapric acid; (4) Poly-DL-α-aminolauric acid.

$$\Delta H^{S} = T \cdot \Delta A \frac{dF}{dT}$$

where F is the surface pressure of transition, ΔH^{S} the change of heat content per ground mole of the surface film, T transition temperature and ΔA the change of area per mole of ground molecule. If we assume a relation between the internal energy of surface film and its heat content, namely

$$H^{S} = U^{S} + FA,$$

we can estimate the change of internal energy of surface film as follows

$$\Delta U^{S} = \Delta H^{S} - F \cdot \Delta A.$$

The calculated changes of enthalpy and changes of internal energy of the phase change of the polypeptide monolayers are shown in Table 2.

Table 2.

	ΔH^{S} (cal./ground mole)	ΔU^{S} (cal./ground mole)
Poly-DL-α-aminolauric acid	567 (25.4°C)	608
Poly-DL-α-aminocapric acid	395 (26.2°C)	440
Poly-DL-α-aminocaprylic acid	285 (25.4°C)	302
Poly-γ-benzyl-DL-glutamate	498 (21.6°C)	565

The difference of internal energy between both states before and after the phase transformation decreases with the length of the side chains of the polypeptide. From the figures in the Table, it may be expected that the energy required for the phase transformation must be very small if the side chains were of four carbon atoms or less. This is caused perhaps by the following reasons. The ratio of two axes of the cross section of the polypeptide molecule perpendicular to the direction of the main chain is not widely different from unity. The thermal energy will readily exceed the energy needed for the transformation. In fact, no plateau was found in the F-A curve of polypeptides of side chains of four carbon atoms such as poly-DL-α-aminocaproic acid, copolymer of glycine-alanine and natural proteins. A report on the monolayer of the copolymer of glycine-alanine, will be given in the following paper in some details. In the cases of polypeptides of short side chains and proteins, the difference of energy between two states may be negligible and reorientation of the molecule occurs without any difficulty. After the reorientation is completed, the film just becomes incompressible and the surface pressure rises suddenly even with a small decrease of area.

It is very interesting that the heat content of the condensed phase of the film is more than that of the expanded phase, contrary to many usual cases met in three dimensions. In the case of polypeptide film, the more condensed phase is of high entropy. The order of the reoriented, condensed film may be worse than that of the film lying flat on the substrate. The order of magnitude of ΔU^S is far less than that of any kind of bonding such as hydrogen linkage. So, the energy change found in the present case is insufficient to break such linkages and may be spent principally for the reorientation.

Recently, Dervichian reported[5] that the plateau would appear in the force-area curve when the film of natural proteins, such as, serum albumin and egg albumin, was compressed relatively rapidly and continuously. Howover, the plateau would disappear from F-A curve if the film was compressed intermittently. He explained his experimental result as follows. There are some modifications in protein film and the transformation between them takes some time, and so, the surface pressure rises if the film is rapidly compressed, before a modification of larger area is transformed to a modification of smaller area.

If our views of reorientation of polypeptide films are accepted and it is assumed that it needs some time for reorientation of the side chains even in the polypeptides of short side chains such as natural proteins, the facts found by Dervichian can be readily explained.

The film of poly-DL-α-aminocaprylic acid gives an intermediate film between that of poly-DL-α-aminocaproic acid or natural proteins and that of the polypeptides which show a distinct plateau in the F-A curve. In this case, the plateau found in F-A curves are not completely parallel to the area axis but somewhat inclined. The less inclined the plateau is, the lower the temperature is. In other words, the more compressible the film at the plateau is, the lower the temperature is. Moreover, the area at which the transformation begins, moves to the smaller area with the rise of temperature, while the area at which the film begins to transform is almost entirely independent of temperature with the polypeptide film of side chains longer than eight carbon atoms or more in length. The points at which the transformation begins to occur lie nearly on a straight line which intercepts the area axis at about 15 Å², as shown in Fig. 3. It may be inferred that the plateau will completely disappear from the F-A curve at about 46°C. and it may be presumed that F-A curve of the film becomes that of poly-DL-α-aminocaproic acid.

As previously mentioned, the plateau appears even in the F-A curve of natural proteins, if the film is compressed rapidly and continuously, for it takes some time to reorient such short side chains found in proteins. In our present experiment, when the area occupied by the film is decreased intermittently. Accordingly, it seems reasonable that the polypeptide such as poly-DL-α-aminocaproic acid which resembles protein shows no plateau in F-A curve.

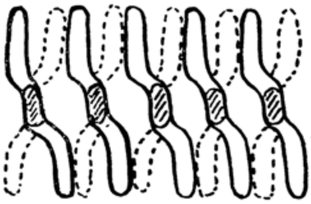

Fig. 6.—Close packed state of DL-polypeptide

To prove our supposition on the reorientation of the polymer, the determination of the thickness of the monolayer is the most direct method. It is expected that a distinct difference will be found between the thickness of monolayers built up at the pressure of more than the pressure at which the plateau ap-

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[5] D. G. Dervichian, *Kolloid-Z.*, **126**, 15 (1952).

pears and that built up at the pressure less than the transition pressure. And this is the case. After the reorientation was completed, the film assumed the shape shown in Fig. 6. With proteins and synthetic polypeptides of short side chains, only the built-up film of this orientation may be obtained. Whereas, with polypeptide films of longer side chains the other type of built-up film in which the side chains lie flat was also obtained.

With poly-DL-α-aminocaproic acid, the thickness of the film was 11.1 Å at pressure as low as 3.1 dynes/cm. In this case, the observed thickness, h_3, is in good agreement with h_1 and h_2. It suggests that the reorientation of the film is already completed. With poly-DL-α-aminocaprylic acid film, the thickness of the monolayer built up at 5.8 dynes/cm. (this pressure is less than the transition pressure) was 9.0 Å, whereas the thickness of the monolayer built up at 9.5 dynes/cm. (this pressure is more than the transition pressure) was 15∼16 Å. This value is in good agreement with h_2. Although similar results were obtained with the monolayers of poly-DL-α-aminocapric acid and poly-DL-α-aminolauric acid, the thickness of the monolayer built up at the pressure higher than the transition pressure was nearly equal to but slightly less than the thickness evaluated by using Stuart models.

(for the most part.) These results support our views on the reorientation of the side chains of polypeptide.

Summary

In the force-area curves of the monolayer of synthetic polypeptides of longer side chains, a plateau which is a region of very high compressibility appears. The appearance of the plateau can be attributed to the reorientation of the polymer molecule at the interface. The pressure at which the plateau appears, decreases with the rise of temperature. From the temperature dependence of this pressure, the heat of transformation was evaluated.

The polypeptides of shorter side chains of four carbon atoms or less in length scarcely need any energy for reorientation. Accordingly, no plateau was found with these polypeptides. The thickness of the monolayer of these polypeptides at the pressure above and under the transition pressure was determined optically by Langmuir-Blodgett technique. The thickness of the monolayer observed was compared with that calculated by using Stuart model (for the most part.) Our views on the reorientatoin of the film were ascertained.

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