The Roles of Prolyl Residue in Polypeptide Monolayers. II. On the Surface Viscosity and Types of Monolayers

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In the preceding paper[1] we have described the effects of prolyl residue in polypeptide monolayers revealed in the surface pressure and potential behavior and deduced their roles in the chain configuration, chain flexibility and intrachain interaction in the polypeptide monolayers. It was found that, as for the polypeptides examined, the presence of prolyl residues makes the monolayers more expanded as their content becomes increased, in spite of the peptide carbonyl groups having the same orientation at the interface. (However, the carbonyl groups in poly-L-proline film are oriented in a different manner.) This expanded nature of monolayer was attributed to the flexible chain configuration of polypeptide arising mainly from the decrease in number of hydrogen bonds. Conversely, the ordinary non-electrolytic polypeptide forms a monolayer of condensed type due to its rigidly held configuration with the strong intrachain hydrogen bonds.

In the study of monolayer the measurement of surface viscosity also gives information concerning its structure and other characteristics. Cumper and Alexander[2], and also Isemura and Hamaguchi[3] established the β-configuration in monolayers of non-electrolytic polypeptides from the surface viscosity measurement. Later, it was noticed[4] that in the monolayer of an amphoteric polypeptide the surface viscosity rises even at the area of low surface pressure if the pH of the aqueous subphase is near the isoelectric point, while the surface viscosity rises at the area of high surface pressure on either side of this pH. Generally, the former behavior appears to be observed in the monolayer of condensed type and the latter in that of expanded type. Such aspects of surface viscosity may be expected in the monolayers of polypeptides with the various proportions and arrangements of prolyl residues and may be related to their chain configuration, chain flexibility and intrachain hydrogen bonding.

In this paper we will report the results of surface viscosity measurements for poly-DL-alanine, poly-L-prolyl-L-leucylglycine and copoly-1:1:1-(L-proline, L-leucine, DL-alanine) and give a further confirmation of inferences obtained from the surface pressure and potential measurements.

Experimental

Materials.—The polypeptide samples were poly-DL-alanine, poly-L-prolyl-L-leucylglycine and copoly-1 : 1 : 1-(L-proline, L-leucine, DL-alanine) which were the same as those reported in the preceding paper[1]. Their degrees of polymerization and spreading solvents were as follows:

	D. P.	Spreading solvent dichloroacetic acid-benzene
Poly-DL-alanine	300	1 : 9
Poly-L-prolyl-L-leucylglycine	13	1 : 4
Copoly-1:1:1-(L-proline, L-leucine, DL-alanine)	129	1 : 4

Methods.—The lower portion of surface pressure was measured by a surface balance of float type and the higher portion (for the other two polypeptides) by a surface balance of hanging plate type. The surface pressures measured by these two balances were ascertained to be identical in the cases of the latter two polypeptides.

Surface viscosity was measured by the damped rotatory oscillation of a disk, utilizing the relation

$$\eta=\frac{2.303I\cdot\Delta\lambda}{2\pi P}\left(\frac{1}{a^2}-\frac{1}{b^2}\right)$$

where I is the moment of inertia of the disk, P the period of oscillation, $\Delta\lambda$ the difference between logarithmic decrements of oscillation in the presence of film and in its absence, a the radius of disk and b the distance between the center of disk and the edge of trough. Usually an outer edge concentric with the disk is put on the aqueous surface in order to calculate surface viscosity by means of the above formula, but its

1) T. Isemura and S. Ikeda, This Bulletin, **32**, 178 (1959).
2) C. W. N. Cumper and A. E. Alexander, *Trans. Faraday Soc.*, **46**., 235 (1950).
3) T. Isemura and K. Hamaguchi, This Bulletin, **27**, 125 (1954).
4) T. Isemura and K. Hamaguchi, ibid,. **27**, 339 (1954).

use makes the reproducibility of measurement unsatisfactory for the monolayer of condensed type such as poly-DL-alanine, because it is difficult for it to flow into the annular region under high compression and it makes a heterogeneous film. It was found that substantially equal values of surface viscosity can be obtained for monolayers of expanded type such as copoly-1 : 1 : 1-(L-proline, L-leucine, DL-alanine) whether the concentric edge is put on or not. Hence, all the measurements were carried out on the surface without concentric edge. In the present apparatus, I was measured to be 43.45 g. cm^2, P 7.50 sec., a 1.28 cm. and b 7.00 cm. and the measurable range of surface viscosity was 10^{-3} to 2×10^{-1} surface poise, where the flow was Newtonian.

To regulate the temperature, a glass serpentine coil was put in the trough, through which water at constant temperature was passed from a thermostat.

Results

The surface pressure-area (Π-A) and surface viscosity-area (η-A) curves of poly-DL-alanine are shown in Fig. 1. The monolayer is of condensed type. Surface

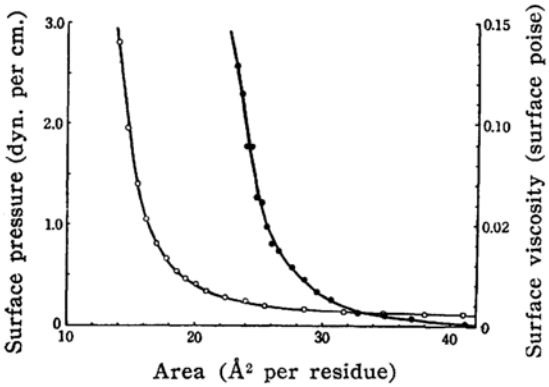

Fig. 1. The surface pressure-area (○) and surface viscosity-area (●) curves of poly-DL-alanine at 9°C.

viscosity begins to rise at the area of 42 Å^2 per residue and gelates at 22 Å^2 per residue and at the surface pressure lower than 0.3 dyn. per cm. Surface viscosity of poly-DL-alanine could be readily measured at room temperature. It appears to be slightly affected by temperature and to increase with the rise of temperature. However, its temperature dependence was not reproducible.

The Π-A and η-A curves of polyl-L-prolyl-L-leucylglycine and copoly-1 : 1 : 1-(L-proline, L-leucine, DL-alanine) are shown in Figs. 2 and 3, respectively. Both films are of expanded type. While their close-packed areas are 57 and 46 Å^2 per three amino acid residues, respectively, both surface

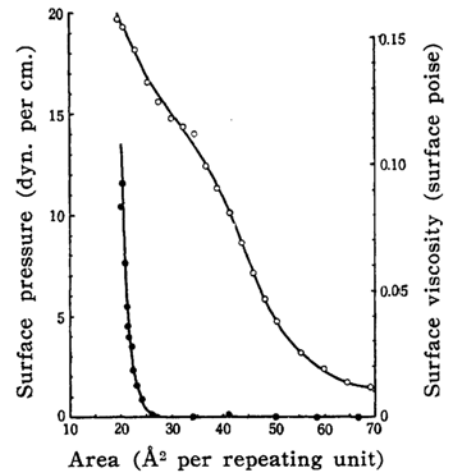

Fig. 2. The surface pressure-area (○) and surface viscosity-area (●) curves of poly-L-prolyl-L-leucylglycine at 16.7°C.

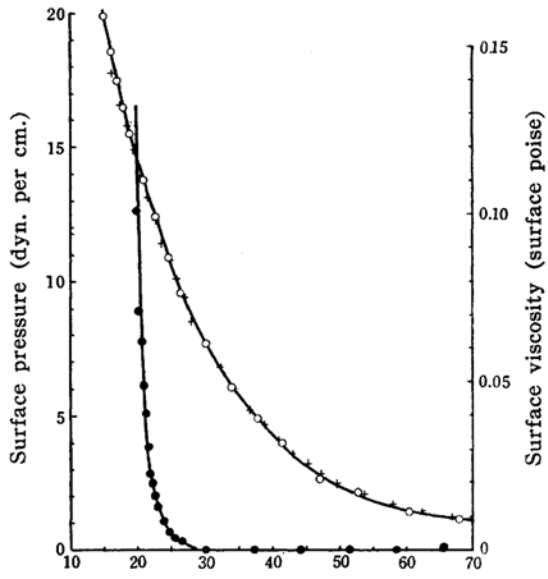

Fig. 3. The surface pressure-area curve (○, at 10.8°C; +, at 26.4°C) and the surface viscosity-area curve (●, at 16.7°C) of copoly-1:1:1-(L-proline, L-leucine, DL-alanine).

viscosities rise at the area less than about 29 Å^2 per three amino acid residues. The corresponding surface pressures range over 15 to 21 dyn. per cm. for the former polypeptide and over 10 to 16 dyn. per cm. for the latter, as the latter film is more expanded than the former. Thus these two polypeptides show almost the same behavior in surface viscosity, irrespective of their different behaviors in surface pressure.

It should be added here that surface

viscosities of these two polypeptides rise at such a small area that films can not occupy an area large enough to spread as complete monolayers. They were initially spread on the surface with an area therefore of about 80 Å^2 per three amino acid residues which is not a sufficiently large area compared with the close-packed areas, and the *Π-A* curves in Figs. 2 and 3, therefore, shift to a smaller area than those obtained when spread on larger areas. The film of copoly-1 : 1 : 1-(L-proline, L-leucine, DL-alanine) was readily compressed even in the high surface pressure region, but the film of poly-L-prolyl-L-leucylglycine required some minutes after compression to establish the final surface pressure.

Surface viscosities of poly-L-prolyl-L-leucylglycine and copoly-1 : 1 : 1-(L-proline, L-leucine, DL-alanine) were found to be markedly dependent of temperature. They were quite reproducible and, to some extent, reversible for compression and expansion. The variation of *η-A* curves with temperature are shown in Figs. 4 and 5, for the respective polypeptide. The higher the temperature is, the lower is the surface viscosity. Both values of surface viscosity and their temperature dependence are almost identical for these two polypeptides. However, as illustrated in Fig. 3, surface pressure was not changed by temperature.

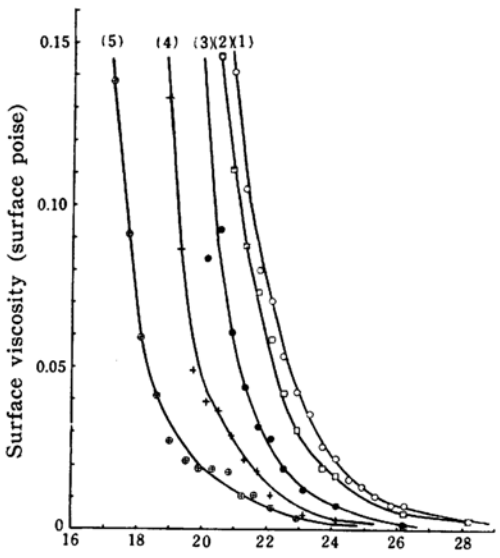

Fig. 4. The variation of surface viscosity-area curves of poly-L-prolyl-L-leucylglycine with temperature. Curves (1) 6.9°, (2) 10.0°, (3) 16.7°, (4) 21.5° and (5) 30.4°C.

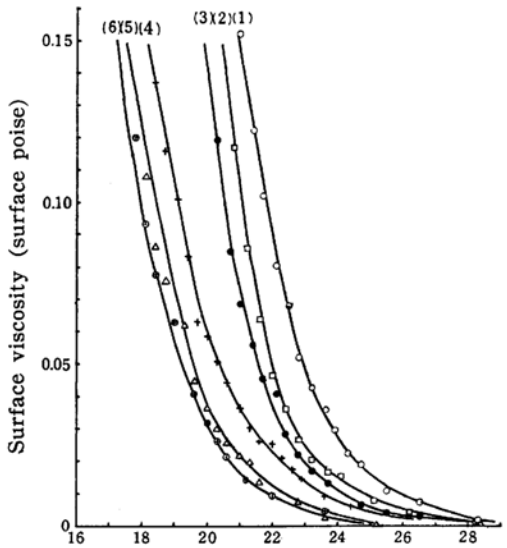

Fig. 5. The variation of surface viscosity-area curves of copoly-1 : 1 : 1-(L-proline, L-leucine, DL-alanine) with temperature. Curves (1) 4.7°, (2) 10.7°, (3) 16.7°, (4) 20.5°, (5) 25.6° and (6) 29.9°C.

Discussion

Surface Viscosity as a Function of Surface Pressure. — It was noticed that surface viscosity of poly-DL-alanine film is already high at the area where surface pressure is sufficiently low and surface viscosities of the other two polypeptide films are detected at the area where surface pressures are considerably high. In other words, surface viscosity of condensed film is actually measured for the monomolecular layer, but that of expanded film is presumably for folded monolayer. Such a difference in surface viscosity was already pointed out by Isemura and Hamaguchi[4] for the film of an electrolytic copolypeptide. It may be attributed to

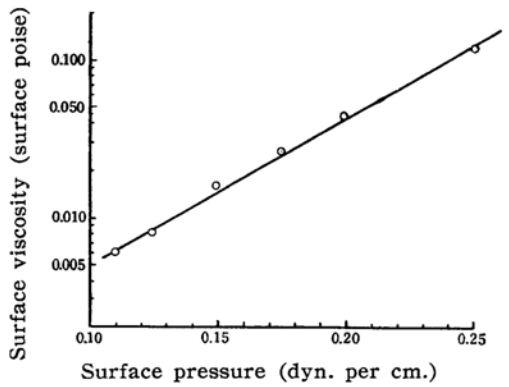

Fig. 6. The log *η-Π* curve of poly-DL-alanine.

the difference in chain configurations of spread polypeptides.

The monolayer of poly-DL-alanine is of condensed type. It was found that for the condensed films of fatty acids[5] and their mixtures[6] the logarithm of surface viscosity is in a linear relation to surface pressure. As shown in Fig. 6, the poly-DL-alanine monolayer follows the same relation as fatty acid films over the region of temperature, 8 to 12°C, and of area, 24 to 34 Å^2 per residue, although the surface pressure at which surface viscosity was measured is much lower. This relation is expressed by

$$\log \eta = \log K + c\Pi \qquad (1)$$

where $\log K$ and c are constants, the values of which are shown in Table I.

TABLE I

	$\log K$	c	B
Poly-DL-Ala	-3.2_3	9.24	0
Poly-L-Pro-L-Leu-Gly	−22.5	0.46	3560
Copoly-(L-Pro, L-Leu, DL-Ala)	−17.3	0.33	3370

On the other hand, poly-L-prolyl-L-leucylglycine and copoly-1 : 1 : 1-(L-proline, L-leucine, DL-alanine) give monolayers of expanded type. It was observed[7] that for the expanded monolayer of polyvinyl acetate the logarithm of surface viscosity changes also linearly with surface pressure at constant temperature. Joly[8] has tabulated the values of surface viscosity as a function of surface pressure for various protein films. These films are probably of expanded type, as they are spread on 0.01 N hydrochloric acid and will be ionized completely. If these values are plotted on a semilogarithm scale, again linear relations are obtained, particularly for the film of gliadin. As shown in Figs. 7 and 8, the films of the two polypeptides also follow the linear relation over the region of temperature, 5 to 30°C, and of area, 21 to 24 Å^2 per three amino acid residues. The relation is written as

$$\log \eta = \log \mu + c\Pi \qquad (2)$$

where c is a constant but $\log \mu$ is a function of temperature, as seen in Table II. Further, the temperature dependence of $\log \mu$ is found to be given by

$$\log \mu = \log K + B/T \qquad (3)$$

where $\log K$ and B are constants, the

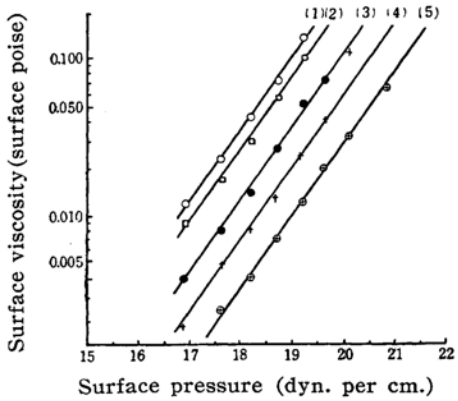

Fig. 7. The $\log \eta$-Π curves of poly-L-prolyl-L-leucylglycine at various temperatures. Curves (1) 6.9°, (2) 10.0°, (3) 16.7°, (4) 21.5° and (5) 30.4°C.

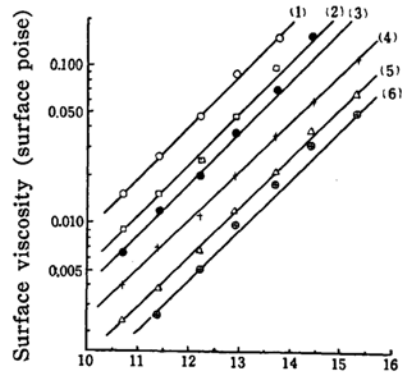

Fig. 8. The $\log \eta$-Π curves of copoly-1 : 1 : 1-(L-proline, L-leucine, DL-alanine) at various temperatures. Curves (1) 4.7°, (2) 10.7°, (3) 16.7°, (4) 20.5°, (5) 25.6° and (6) 29.9°C.

TABLE II

Poly-L-Pro-L-Leu-Gly			
T (°C)	$\log \mu$	c	
30.4	−10.80	0.46_4	
21.5	−10.54	0.45_7	$\log K = -22.7_0$
16.7	−10.21	0.46_3	$B = 3610$
10.0	−9.89	0.46_1	
6.9	−9.76	0.46_6	
Copoly-(L-Pro, L-Leu, DL-Ala)			
T (°C)	$\log \mu$	c	
29.9	−6.29	0.32_4	
25.6	−6.09	0.32_2	
20.5	−5.80	0.31_7	$\log K = -17.2_7$
16.7	−5.76	0.33_3	$B = 3340$
10.7	−5.48	0.32_0	
4.7	−5.42	0.33_5	

5) G. E. Boyd and W. D. Harkins, *J. Am. Chem. Soc.*, **61**, 1188 (1939).
6) G. E. Boyd and F. Vaslow, *J. Colloid Sci.*, **13**, 275 (1958).
7) T. Isemura and K. Fukuzuka, *Mem. Inst. Sci. Ind. Res., Osaka Univ.*, **13**, 137 (1956).
8) M. Joly, "Surface Chemistry", But-terworths Scientific Publications, London (1949), p. 157.

values of which are also shown in Table II.

Surface Viscosity as a Function of Temperature. — As mentioned before, surface viscosity of poly-DL-alanine monolayer scarcely varies with temperature but tends to increase slightly as temperature is raised. This appears to be a general aspect of the monolayer of condensed type.

For the films of poly-L-prolyl-L-leucylglycine and copoly-1 : 1 : 1-(L-proline, L-leucine, DL-alanine) surface viscosities diminish as temperature rises and it was found that plots of logarithm of surface viscosity against the inverse of temperature are linear over the region of temperature, 5 to 30°C, and of areas, 21 to 24 Å^2 per three amino acid residues, as illustrated in Figs. 9 and 10. That is, the relation is fitted to the Andrade equation

$$\log \eta = \log \nu + B/T \qquad (4)$$

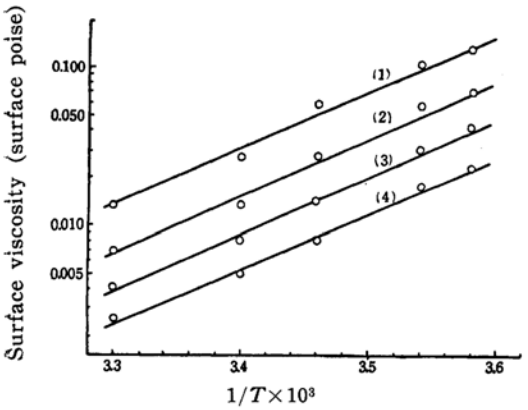

Fig. 9. The $\log \eta - 1/T$ curves of poly-L-prolyl-L-leucylglycine at various areas (at various surface pressures). Curves (1) 21.0 Å^2, (2) 22.0 Å^2, (3) 23.0 Å^2 and (4) 24.0 Å^2.

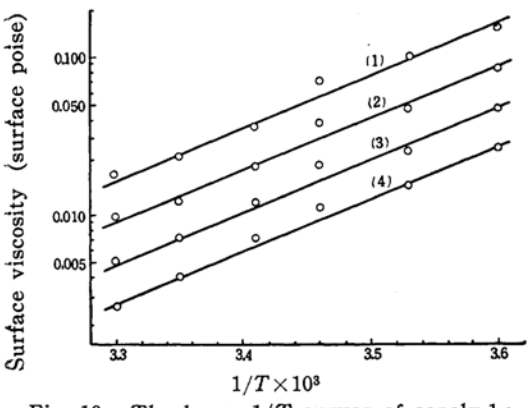

Fig. 10. The $\log \eta - 1/T$ curves of copoly-1 : 1 : 1-(L-proline, L-leucine, DL-alanine) at various areas (at various surface pressures). Curves (1) (21.0Å^2, (2) 22.0 Å^2, (3) 23.0 Å^2 and (4) 24.0 Å^2.

TABLE III

Poly-L-Pro-L-Leu-Gly

Π (dyn./cm.)	$\log \nu$	B	
19.2	−13.61	3520	
18.7	−13.92	3580	$\log \nu = -22.4_8$
18.2	−14.12	3610	$c = 0.46$
17.6	−14.38	3500	

Copoly-(L-Pro, L-Leu, DL-Ala)

Π (dyn./cm.)	$\log \nu$	B	
13.7	−12.88	3320	
12.9	−13.14	3370	$\log \nu = -17.3_0$
12.4	−13.41	3390	$c = 0.32$
11.4	−13.66	3390	

where B is a constant but $\log \nu$ depends on surface pressure. This has already been established for films of polyvinyl acetate[9] and 6-nylon[10], although the dependence of $\log \nu$ on surface pressure is not explicitly indicated. The values of B and $\log \nu$ are given in Table III. It can be seen that $\log \nu$ is represented by

$$\log \nu = \log K + c\Pi \qquad (5)$$

as a function of surface pressure, where both $\log K$ and c are constants the values of which are also shown in Table III.

From the data given in Tables II and III, numerical coincidences may be noticed between $\log K$ in Eqs. 3 and 5, between c in Eqs. 2 and 5 and between B in Eqs. 3 and 4. Consequently, the surface viscosity equation is reduced to

$$\log \eta = \log K + c\Pi + B/T \qquad (6)$$

for the films of poly-L-prolyl-L-leucylglycine and copoly-1 : 1 : 1-(L-proline, L-leucine, DL-alanine). Values of constants are listed in Table I. The values of B for these two polypeptides are found to be almost equal to each other, which should be the necessary condition for the equal temperature coefficient of surface viscosity.

Configuration and Flexibility of Polypeptide Chains. — In comparing the close-packed area with the area where surface viscosity was measured, it will be evident that surface film in this region corresponds to liquid or melt in bulk rather than to dilute solution, and accordingly, surface viscosity to viscosity of liquid or melt. It is generally accepted that a transition takes place at the glass temperature or the second-order transition temperature for polymers, below which the segmental motion becomes frozen-in. The segmental motion is exhibited in the behavior of

9) T. Isemura and K. Fukuzuka, *Mem. Inst. Sci. Ind. Res., Osaka Univ.*, **14**, 169 (1957).
10) K. Inokuchi, This Bulletin. **28**, 453 (1955).

melt viscosity[11]. At the transition temperature melt viscosity decreases markedly with the rise of temperature. While above the transition temperature melt viscosity alters with temperature in such a way as expressed by the Andrade equation, below that temperature it is not influenced by temperature very much but rather depends on other complicated factors[12]. The temperature at which such freezing-in of the segmental motion will occur depends on the structural factors such as inter- or intrachain interaction, chain flexibility or steric effect. Then at a given temperature a polymer will behave as liquid or glass whether that temperature is higher or lower than the glass temperature. This idea can be adequately applied to explain the observed behavior of surface viscosity of monolayer. Thus the monolayers of expanded type and condensed type might be correlated reasonably with a melt and glassy state in bulk, respectively. In other words, a spread polymer which gives a film of expanded type will behave as an ordinary liquid at the temperature where film properties are measured, since the segmental interaction is weak and the molecular chain is flexible; also the segmental motion of a spread polymer in a film of condensed type is inhibited at that temperature, since the chain is rigidly held owing to the strong segmental interaction. The difference is revealed most distinctly in surface viscosity behavior.

Eyring et al.[13,14] derived the surface viscosity equation by a simple analogy with liquid viscosity, regarding monolayer as a two-dimensional liquid. According to them, surface viscosity is expressed by

$$\eta = \frac{h}{A_f} \exp\left(\frac{\Delta F^*}{kT}\right) \tag{7}$$

where h is the Planck constant, k the Boltzmann constant and A_f is the area occupied by a unit of flow. The free energy of activation for flow per unit, ΔF^*, is given by

$$\Delta F^* = \Delta E^* + \Pi \Delta A^* - T\Delta S^* \tag{8}$$

where ΔE^* and ΔS^* are the energy and the entropy of activation per unit, respectively, and ΔA^* is the increment of area in the activated state over that of the initial state. Substituting Eq. 8 into Eq. 7, the final equation becomes

$$\ln \eta = \ln \frac{h}{A_f} - \frac{\Delta S^*}{k} + \frac{\Delta A^*}{kT}\pi + \frac{\Delta E^*}{kT} \tag{9}$$

This equation has the same form as Eqs. 1 and 6. From their comparison it can be seen that

$$\Delta E^* = 2.303\, kB \tag{10}$$

$$\Delta A^* = 2.303\, kTc \tag{11}$$

$$\frac{h}{A_f} \exp\left(-\frac{\Delta S^*}{k}\right) = K \tag{12}$$

Thus ΔE^* and ΔA^* may be calculated from the data in Table I. Although the value of ΔS^* can not be determined uniquely from a single experimental value of log K, its approximate value may be assigned, as it is not very sensitive for the choice of values of A_f. Table IV shows these data for the polypeptide films studied in the present investigation.

TABLE IV

	ΔE^* (kcal./mol.)	ΔA^* (Å²)	ΔS^{**} (e. u.)
Poly-DL-Ala	0	8000	−110∼−120
Poly-L-Pro-L-Leu-Gly	16.3	420	−21∼ −29
Copoly-(L-Pro, L-Leu, DL-Ala)	15.4	300	−45∼ −50

* Values when A_f is assumed to be 10 Å² to ΔA^*

For the monolayer of poly-DL-alanine, ΔE^* was found to be nearly zero. This suggests that the flow mechanism is different from that in liquid state and the polypeptide molecules flow under shear by breaking the entanglement or linkages such as van der Waals or hydrogen bonds between them. This may be expected from the discussion above that such a flow occurs for the film of polypeptide in which the segmental interaction is strong and the rigidity of the chain is high. This result is consistent with that deduced for the condensed nature of this film in the previous paper[1]. Further support is provided by estimating the value of ΔA^*, which was found to be about 8000 Å² per flow unit. Since the close-packed area of poly-DL-alanine is 15 Å² per residue, the area obtained above is occupied by about 500 residues. Taking account of the ambiguity concerning the degree of polymerization of material, 300, it may be roughly assumed that a unit of flow is composed of a poly-DL-alanine molecule. In addition, a large

11) T. Alfrey, G. Goldfinger and H. Mark, *J. Appl. Phys.*, **14**, 700 (1943); E. Jenkel, *Kolloid-Z.*, **120**, 160 (1951).
12) K. Ueberreiter and H. J. Orthmann, ibid., **126**, 140 (1952).
13) E. J. Moore and H. Eyring, *J. Chem. Phys.*, **6**, 391 (1938).
14) S. Glasstone, K. J. Laidler and H. Eyring, "The Theory of Rate Processes", McGraw-Hill Co., New York (1941), p. 561.

negative value for ΔS^*, -120 e. u., was assigned to the polypeptide. In view of the deduction concerning dependence of the entropy of activation for flow in bulk on chain length of polymers[15] and the result on surface viscosity of polyvinyl acetate[7], the entropy of activation for flow in film might be scarcely influenced by the degree of polymerization of polymers, that is, the term involving the degree of polymerization contributes to the entropy only a few e. u. Then the large negative value for ΔS^* should be characteristic of the monolayer of poly-DL-alanine. It might be attributed to the highly disordered configuration of flow unit or molecule of poly-DL-alanine at the interface. Probably the spread molecule is coiled and held together in monolayer.

On the other hand, for the films of poly-L-prolyl-L-leucylglycine and copoly-1 : 1 : 1-(L-proline, L-leucine, DL-alanine) values of ΔE^* are not zero but 16.3 and 15.4 kcal. per mole, respectively, and nearly equal to each other. These are of comparable order of magnitude with those reported, namely, 12.7 kcal. per mole for the film of polyvinyl acetate[9] and 6.4 kcal. per mole for the film of 6-nylon[10], although the sizes of their unit of flow will be different from one another. But the larger values for these two polypeptides than the other might come from the additional energy necessary for breaking (more) hydrogen bonds when units of flow move. The values of ΔA^* were 420 and 300 Å^2 per three amino acid residues, respectively. The size of flow units can not be estimated in the same way as above, as the viscous flow takes place at such small areas that they do not always correspond to a monomolecular layer. The polypeptide chains are probably in a folded configuration partly submerged into the aqueous subphase in these areas. The small negative values of ΔS^*, -25 and -50 e. u., respectively, would be an indication of the relatively regular configuration of chains. Both the mechanism of flow through the activation process and the regular folding of chains under compression favor the weak interaction and the flexibility of chains. This supports the results obtained in the previous paper[1].

All the differences in surface viscosity behavior between poly-DL-alanine and the other two polypeptides are based on the types of monolayers, namely, the nature of condensation and expansion of films. The types of monolayers depend on the structural factors defined by the proportion and arrangement of prolyl residues in polypeptide chains. But the surface viscosities and their temperature dependences were found to be equal to each other for poly-L-prolyl-L-leucylglycine and copoly-1 : 1 : 1-(L-proline, L-leucine, DL-alanine), which will originate from their identical chain configurations, irrespective of their different amino acid sequences in chains. The fact that the surface pressure of the former polypeptide is higher than that of the latter and shows some time effect, will mean that the former chain folds itself by compression with greater difficulty, as described in the preceding paper[1].

15) W. Kauzmann and H. Eyring, *J. Am. Chem. Soc.*, **62**, 3113 (1940). See also Ref. 14, p. 501.

Summary

To investigate the roles of prolyl residue in polypeptide monolayers, surface viscosity was measured for the monolayers of poly-DL-alanine, poly-L-prolyl-L-leucylglycine and copoly-1 : 1 : 1-(L-proline, L-leucine, DL-alanine).

For the monolayer of poly-DL-alanine which is of condensed type, the surface viscosity was found to rise at the area larger than the close-packed area and scarcely to be dependent of temperature. This result was correlated with that observed in the glassy state of polymers in bulk and interpreted by assuming that the polypeptide chain is rigid owing to the strong interaction. For the films of the other two polypeptides which are of expanded type appreciable surface viscosities were manifested at the areas much smaller than their close-packed areas and temperature exerted marked influence on surface viscosities. Their chain configurations were considered to be flexible owing to the weak interaction, since the flows in films occur as in melt of polymers in bulk.

Further evidence for this inference was obtained from the results on dependences of surface viscosity on surface pressure and temperature. The dependences were found to follow Eyring's equation of surface viscosity. It was found that in the monolayer of poly-DL-alanine a unit of flow is roughly composed of a molecule and its chain is rather coiled at the interface, but the chains of the other two polypeptides are in a regularly folded configuration partly submerged into the

aqueous phase when compressed, as they are flexible.

The difference in surface viscosity behavior between poly-DL-alanine and the other two polypeptides was related to the condensed and expanded nature of films, which is defined by the content and distribution of prolyl residues in polypeptide chains. It was, however, observed that poly-L-prolyl-L-leucylglycine and copoly-1 : 1 : 1-(L-proline, L-leucine, DL-alanine) exhibit the same surface viscosity behavior, irrespective of their different distribution of prolyl residues in chains, while the former has a higher surface pressure than the latter.

All the results obtained were found to confirm the view deduced from the surface pressure and potential measurements.

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