The Structure of Surface Denatured Protein. V. The Surface Film of Diketo-piperazine

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Introduction

Many investigations have been made about the properties of monolayers of proteins. Rideal[1] and Alexander[2] insist that the unfolded polypeptide chain has β-keratine configuration, while Isemura[3] has explained his results by using Mizushima and Shimanouchi's model. But none has regard for the existence of diketopiperazine configuration on the surface. It may not be probable that polypeptide chain has diketopiperazine configuration on the water surface. Yet, the existence of diketopiperazine configuration cannot be denied until the limiting area of diketopiperazine film is determined.

The author has investigated the diketopiperazine monolayer and obtained the value of limiting area from the results of F—A, V—A and η—A curves.

Experimental

Material—Diketopiperazine is prepared from glycine by Dr. Tadao Sugita, in the Institute of Chemistry, Faculty of Science, University of Tokyo, and is recrystallized several times. This is spread on the surface of KCl solution of about 0.2 N concentration from the solution in the mixed solvent of dichloracetic acid and benzene. The spread film is uniform as checked by the constancy of surface potential within the error of 1 mV.

Measuring Apparatuses—Surface pressure was measured by a modified Wilhelmy balance, which has been described in the Part I of this series[4]. Surface potential is measured by the apparatus described in Part III of this series[5]. Viscosity of monomolecular film can be measured by the method of flowing out through a capillary canal[6], by the method of the oscillation of a ring in the monolayer[7] or by the method of rotation of a ring (analogous to a cell of Couette) in the monolayer[8].

The apparatus and the procedure of the present measurements are essentially the same as those described by Joly[6]. A diagram of the apparatus used is shown in Fig. 1. This consisted essentially of a brass tray T, containing a canal C and two Wilhelmy balances W and W'. The canal is 1.1 cm long and 0.062 cm broad, and have door D at its end, which controls the flow of monomolecular film. Harkins[9] gave for this case the following equation,

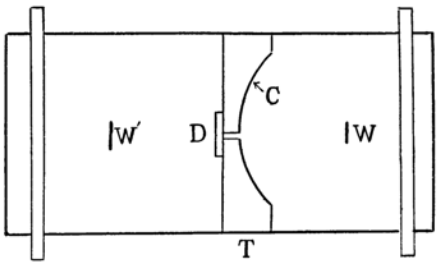

Fig. 1

$$\eta = \frac{\alpha}{Q} \cdot \frac{d^3}{12} - \frac{d \cdot \eta_0}{\pi}$$

where α is the film pressure gradient, Q the quantity of the film which passes through the canal in unit time, d the breadth of the canal and η_0 the viscosity of the substrate.

Thus, the surface viscosity η can be determined by the measurement of the quantity Q.

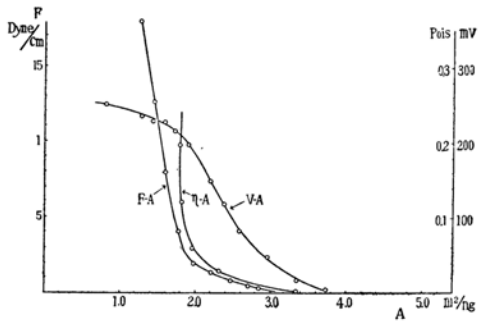

Fig. 2

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3) T. Isemura and K. Hamaguchi, This Bulletin, **25**, 40 (1952).
4) K. Imahori, This Bulletin, **25**, 7 (1952).
5) K. Imahori, This Bulletin, **25**, 13 (1952).
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7) L. Fourt, *J. Phys. Chem.*, **43**, 887 (1939).
8) R. Chaminade, D. G. Dervichian et M. Joly, *Journ. Chim. Phys.*, **47**, 883 (1950).
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Results

The F—A curve in Fig. 2 shows the plot of the film pressures in dyne per centimeter against the film areas in square meters per milligram. The measurements were done as carefully as possible. The film was compressed slowly with small decrements. Ample time was allowed after each decrease in area for the attainment of equilbrium surface pressure. The result shows that the limiting area of this curve is 1.85 m^2/mg.

The V—A curve in the figure shows the plot of the surface potentials of the film in millivolt against the film areas in square meters per milligram. The curve shows that the limiting area is 1.82 m^2/mg.

The η—A curve in the figure shows the plot of the surface viscosity in pois against the film area in square meters per milligram. All the experiments, represented by the circlets in the figure, were carried out under surface pressure differences below 2.5 dyne per centimeter. In the figure, sudden increase in slope of the curve occured at 1.82 m^2/mg and this shows that the limiting area is 1.82 m^2/mg.

Discussions

The above three curves show that the limiting area of diketopiperazine monomolecular film is 1.82—1.85 square meters per milligram. The mean of the three values is 1.83 m^2/mg (or 34.6 $Å^2$ per one diketopiperazine molecule).

The crystal structure of diketopiperazine has been determined by Corey[10] by X-ray diffraction. From this result, the limiting area per one diketopiperazine molecule is to be 35.5 $Å^2$ when diketopiperazine molecule are orientated flat on the surface (the broad side on) perhaps in such a way as shown in Fig. 3.

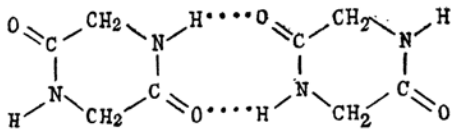

Fig. 3

Comparing this result with that obtained by author's experiment, we can say the following: The diketopiperazine molecules lie flat when they are expanded on the water surface. But when the film is compressed below the limiting area, diketopiperazine molecules probably begin to rise on the surface.

On the other hand, the author has obtained the limiting area of the horse serum albumin molecule to be about 9820 $Å^2$ [4]. The chemical constitution of the serum albumin molecule has been determined by several authors[11] and it is calculated that one serum albumin molecule contains about 780 amino acid residues. Thus, the mean limiting area per one amino acid residue becomes to be 12.5 $Å^2$. This value is far less than the limiting area of the diketopiperazine film (17.3 $Å^2$/amino acid residue) and, it can be denied that the polypeptide chain of the serum albumin shows diketopiperazine configuration as it is spread on the surface.

The value of 14.7 $Å^2$ is obtained by Isemura[3] for synthetic protein analogues and also by Alexander for the alanine-polymer[2]. And it is obvious that synthetic protein analogues also does not show the diketopiperazine confiuration, when they are spread on the water surface.

As Isemura has insisted, the above value of 14.7 $Å^2$ can be explained most perfectly by using Mizushima and Shimanouchi's E-type model,[12] while one amino acid residue of B-type molecule would cover about 10 $Å^2$. Dr. T. Shimanouchi and the present author has ascertained these two values from the experiments of glycine-polymer monolayer, which will be published in other report.

Anyhow, from these values, the configuration of polypeptide chain of surface denatured serum albumin does not comprise the diketopiperazine ring. Probably, it is the mixture of B-and E-type of Mizushima and Shimanouchi's model[12].

Summary

1. The curves of the surface pressure against area, the surface potential against area, and surface viscosity against area were obtained with diketopiperazine. All the three curves show the limiting area to be about 1.83 m^2/mg or 34.6 $Å^2$ per molecule.

2. Comparing this result with that obtained by Corey, diketopiperazine molecules are thought to lie flat on the surface.

3. The above value is far larger than the limiting areas of native serum albumin or synthetic protein analogues. And the polypeptide chain will not show the diketopiperazine configuration on the surface.

The author wishes to express his hearty thanks to professors S. Mizushima and B.

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