The Structure of Surface-Denatured Protein. III. Determination of the Shape of Surface-Denatured Horse Serum Albumin

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In the first paper[1] of this series, the molecular weight and the surface area covered by one protein molecule were reported. In the second paper,[2] theoretical treatment on the relation between the shape of the protein molecule and the surface diffusion constant has been given.

In the present paper, the method of calculating the surface-diffusion constant from the measurement of surface potential as well as its result will be reported.

Apparatus of Surface Potential Measurement

There are two methods of measurement of surface potential. One is the so-called vibrating plate method,[3] by Zisman, and the other is the ionization method adopted by Rideal and others.[4] Recently, several chemists in this country have constructed the apparatus which in all cases is of the former type. The present author has constructed one of the latter type, principally because the latter type is superior to the former in the experiment of surface diffusion, as the electrode of the latter type is so thin that the surface potential can be measured from point precisely. The apparatus is shown in Fig. 1.

In the figure, T is the trough, filled with water, upon which the protein film under investigation is spread. The trough is held on a bakelite stand, which is insulated from the earth electrically. The potential of T can be varied by potentiometer P, which is connected with T through caromel cell C and salt junction J. A is an air electrode —made of platinum wire of about 1 mm. diameter and coated with polonium. The polonium ionizes the air between A and T and conncets A electrically with the top of the surface film. A is supported by a pyrex glass bar which is fixed on a steel stand and can be freely moved vertically and horizontally. U is a vaccum tube, UX 54 made by the Tokyo Shibaura Electric Co. Ltd., which has a very large grid resistance ($10^{10}\Omega$). The filament current is 200 mA. R_1, R_2 and R_3 are all variable resistances of 25 Ω. R_0 and R_0' are also variable resistances of 500 kΩ and 100 Ω respectively. R_g is also a variable resistance, the value of which is just equal to the critical damping

(1) K. Imahori, This Bulletin, **25**, 7 (1952).
(2) K. Imahori, This Bulletin, **25**, 10 (1952).
(3) W. A. Zisman, *Rev. Sci. Instr.*, **3**, 367 (1932).
(4) J. H. Schulman and E. K. Rideal, *Proc. Roy. Soc.*, **130**, A, 259 (1931).

reistance of the galvanometer used, and equal to 1 kΩ in the present case.

R_a anc R_b are fixed resistances of 20 kΩ and 1 kΩ respectively. I_f is a milliammeter having a scale up to 500 mA. and G a galvanometer, sensitivity of which is about 2×10^{-5} A. The apparatus must be completely shielded electrically. The operation of this apparatus is as follows.

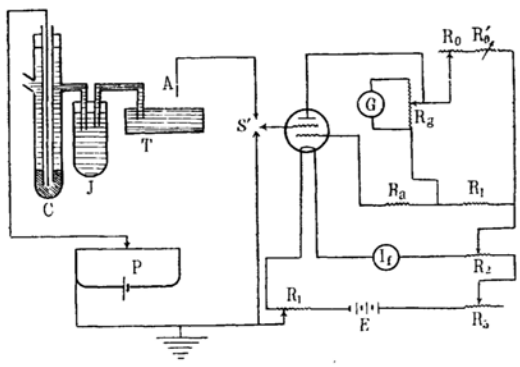

Fig. 1.—Apparatus for measuring the surface potential.

First the switch S in Fig. 1 is put downward and the grid is earthed. By adjusting R_0 and R_0', the needle of G is set to its zero point. Then A is brought about 1~2 mm. above the cleaned surface of the water contained in the trough T. S is switched upward and A is connected to the grid. The needle of the G moves then far from zero point and it is brought back again to near zero point by adjusting P. Now, S is put downward, then upward again and P is adjusted and this operation is repeated several times until the needle of G does not move by this operation. The value, which P indicates, is just equal to the contact potential between A and the water surface and can be expressed by V_1.

Then monomolecular film of protein is put on the water surface. The contact potential between A and water carrying the protein film can be measured by the similar way and is expressed as V_2. The surface potential ΔV of the protein film is equal to the difference of V_2 and V_1, or

$$\Delta V = V_2 - V_1.$$

The Relation between Concentration and Surface Potential

In the experiment of the surface diffusion constant, it is needful to measure the concentration of protein on the surface. There are probably only a few ways to measure this concentration. The present author has done this from the measurement of surface potential, because, in the region of low concentration, surface potential is thought to be proportional to surface concentration. This can be ascertained from the following experiments. The curve V_P in Fig, 2 shows the relation between the surface potential and the surface cencentration of serum albumin, while the curve F_P shows the relation between the surface pressure and the surface concentration. From these two curves it becomes evident that the surface potential is proportional to the surface concentration in the region of concentration below 0.1 mg./m.^{2} or in the region of surface pressure below 0.05 dyne/cm. Similarly, curves V_C and F_C show that the surface potential of castor oil monolayer is proportional to the surface concentration in the region of surface pressure below 0.5 dyne/cm.

When the mixed film of castor oil and protein monolayer is expanded on the surface, the surface potential varies according to the curve V_m in Fig. 2, where the amount of protein used is constant——2.085×10^{-7}g., while the amount of castor oil is varied.

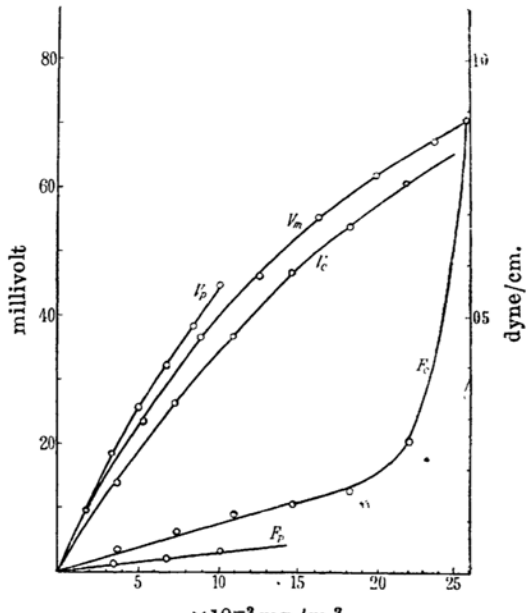

Fig. 2.

From this curve it is also evident that the surface potential of the mixed film becomes just the sum of two partial surface potentials, namely, the surface potentials of two components expanded on the same surface area, each alone respectively. This shows that there is very little interaction between molecules of protein and castor oil on the surface, under very low surface pressure.

Mesurement of the Surface Diffusion Constant

As is shown in Fig. 1, a tray of 60 cm. long,

20 cm. broad, and 4 cm. deep, is filled with distilled water or other aqueous substrates. The surface of the water is cleaned with a moving barrier (M and M′ in Fig. 3) and then is divided into two

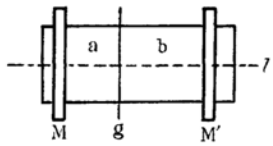

Fig. 3.

parts by a very thin glass rod (g), which is so thin and so light that it can be pushed away by even the slightest surface pressure. The contact potential V_1 of the water surface and the electrode is measured by the apparatus mentioned above. Serum albumin solution in water of 0.0209% concentration is put on one side (side a), of the water surfece, from a micrometer syringe, which makes the gaseous film of serum albumin, and the rod g is pushed to the other side (side b). The sample of serum albumin is just the same as that used in the first paper of this work.

Then a few drops of very dilute benzene (or ethylether) solution of castor oil are put on both sides of the rod until the surface pressures of the both sides balance with each other and the rod g comes to an equilibrium position. The balanced surface pressure must be less than 0.5 dyne/cm.

The air electrode A is carried along the line l of Fig. 3. The surface potential along l can be

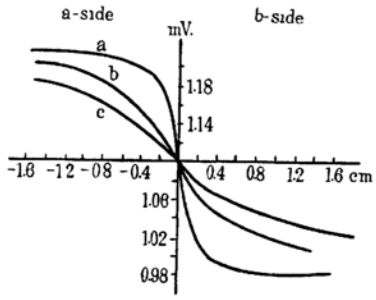

Fig. 4.—Surface potential curve.

shown schematically as the curve a in Fig. 4, in which the abscissa shows the the distance along l. The zero point corresponds with the point where the glass rod crosses the line l. The glass rod must be kept perpendicular to l.

Now, the glass rod is taken away from the water surface and the protein begins to diffuse into the b-side. If, however, castor oil had not been put on the b-side, the protein molecule would flow on the water surface just as a gas flows from a vessel of higher pressure into one of lower pressure, and soon all the surface will be covered by a homogeneous protein film. The velocity of such flow would not mean the true surface diffusion velocity. To avoid the error from this flow movement, some substance (such as castor oil) is put on the b-side so as to balance the surface pressures of both sides. And thus, the net diffusion constant on the surface can be determined.

As time elapses, the surface potential gradient at zero point becomes gradually less and less just as is shown schematically by the curves b and c in Fig. 4.

As is cited above, the experiment is carried out at low surface pressure, and under this condition the potential gradients at various points are proportional to the gradients of the concentration of protein molecules, just as is shown in the above section. The surface potential along l after 24 hours of diffusion is shown in Fig. 5 and the gradients at various points of this curve are shown in Fig. 6. The measurement can be carried out within the error of 0.5 mV.

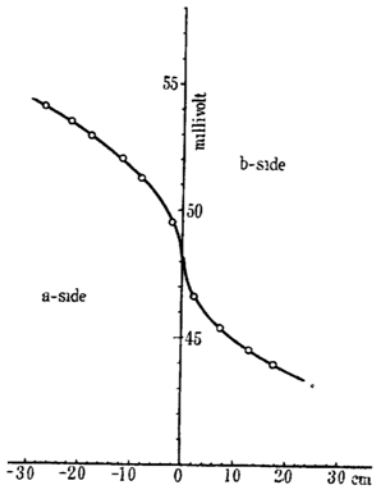

Fig. 5.

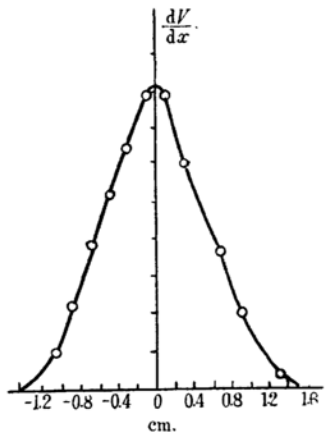

Fig. 6.—Surface potential gradient.

The curve in Fig. 6 also represents the gradients of the concentration of protein on

the surface at various points, so that, just as in the case of three-dimensional diffusion, the diffusion constant can be calculated by the inflection point method. If the distance from the zero point to the inflection point is expressed as μ and the time elapsed from the initiation of the diffusion to the measurement is expressed by t, the diffusion constant can be given as

$$D=\frac{\mu^2}{2t} \qquad (1)$$

From Fig. 6 and Eq. (1), and also by using the data of $\mu=0.14$ cm., D is calculated to be 1.1×10^{-6} cm.^{2}/sec. By putting this value into Eq. (11) given in the second report of this investigation,

$$M=\frac{9dR^2T^2}{256\eta^2ND^2}(f_0/f)^2$$

and also by using the data, V (reciprocal of the density of protein)$=0.748$,[5] d (thickness of the film)$=10$ Å.,[6] M (molecular weight of protein)$=70{,}400$,[1] f/f_0 (friction ratio) is given as 1.28. Using the relation shown in Fig. 1 of the same report mentioned above, the ratio of two axes turns out to be 8.0.

As was shown in the first report, the surface area covered by one serum albumin molecule is 9800 Å.^{2} and thus the length of the two axes are calculated as: $a=20$ Å. and $c=160$ Å. Therefore, the shape of the surface-denatured serum albumin molecule will be schematically expressed as in Fig. 7.

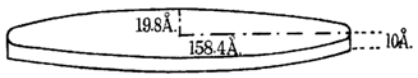

Fig. 7.—Shape of the surface-denatured serum albumin molecule.

(5) T. Svedberg, "Ultracentrifuge," 1940.
(6) K. Imahori and Y. Yoneyama, unpublished.

Summary

1. The apparatus for measuring surface potential by the so-called ionization method has been described.

2. The relation between the surface potential and concentration of serum albumin and castor oil monolayers have been obtained.

3. The method for determining the shape of surface-denatured protein molecules has been described. This can be carried out on the grounds of the determination of surface-diffusion constant by the surface potential measurement.

4. The shape of horse serum albumin has been decided to be an elliptic disk having the value of $a=20$ Å., $c=160$ Å. and thickness of 10 Å.

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