The Structure of Surface-Denatured Protein. I. Molecular Weight and Surface Area of Horse Serum Albumin Molecule

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As Bull and others have shown, it is possible to obtain a very thin protein film of the thickness of the order of 10 Å. by putting a small quantity of powdered protein or protein solution on the surface of concentrated salt solution[1] or water solution of propyl alcohol.[2] The protein molecule in such a film may lose its original globular shape; it is considered that the polypeptide linkage has the extended, to become the so called β-form, This can be shown by transferring the film to the surface of a solid and taking its photograph by x-ray diffraction.[3] The transformation from α-form to β-form is characteristic for the denaturation phenomenon. Therefore, the above-mentioned protein film is sometimes called surface-denatured protein. Neurath[4] has pointed out, that the surface denaturation is the most perfect denaturation. And it is quite interesting to investigate the structure of the surface-denatured protein as well as the mechanism of the surface denaturation.

This problem has been investigated by the present author from several points of view and in this paper the molecular weight and surface area of the surface-denatured horse serum albumin will be reported. The former was determined by H. B. Bull's method, but the latter by the measurement of the compressibility of the film.

Surface Balance

The author modified the so-called Wilhelmy[5]

(1) H. B. Bull, *J. Am. Chem. Soc.*, **67**, 4 (1946).
(2) H. Theorell, *Trans. Faraday soc.*, **35**, 1413 (1939).
(3) W. T. Astbury, F. O. Bell, E. Gorter and J. van Ormondt, *Nature*, **142**, 33 (1938).
(4) H. Neurath, J. P. Greenstein, F. W. Putnam and J. O. Erickson, *Chem. Rev.*, **34**, 157 (1945).
(5) Wilhelmy, *Ann. Physik.*, **119**, 177 (1863).

type surface balance and has used it instead of the Adam's type. One of the bowls of the chemical balance (made by E. Sartorius in Germany) is taken away, and a long metal wire is hung instead. A very thin glass plate is hung on the tip of the wire. The glass plate must by kept in the chromic acid mixture; it should be washed with distilled water and then dried before use, for even the slightest stain on the plate will make the measurement quite incorrect.

The length of the wire is controlled so that about one third part of the plate may be immersed under the water surface. An extremely thin glass needle is put on the tip of the needle of the balance. A scaled microscope of 40 magnification is fixed in front of the needle and is brought to its focus.

Then, putting some balance-weight on the bowl, the balance is brought in equilibrium and the position of the glass needle on the scale of the microscope is read. When some film is put on the water surface, its surface tension decreases, and the glass plate rises a little. The needle then moves to a new equilibrium position and the deflection of the needle ΔQ_1 can be expressed as

$$\Delta Q_1 = k\Delta\gamma. \tag{1}$$

Here, $\Delta\gamma$ is the surface pressure, k is a constant indicating the sensivity of the balance, contact angle being assumed to be zero. Then the balance weight on the bowl is decreased so as to bring the needle to its original position. If the decrease in weight is expressed as ΔG, the following equation must hold.

$$g\Delta G = 2(t+b)\Delta\gamma \tag{2}$$

Here, g is the gravity constant and t the thickness and b the width of the plate respectively. And thus the surface pressure can be calculated from (2) or the following relation, which can be derived from (2).

$$\Delta\gamma = \frac{g\cdot\Delta G}{2(t+b)} \tag{3}$$

In the present experiment, $t = 2\times10^{-3}$ cm. and $b = 5.5$ cm.

But it is troublesome to bring the needle just to the original position by adjusting the weight on the bowl. In such a case, the surface pressure can be calculated as follows.

When the weight on the bowl is decreased by ΔG_1 and the needle is brought to the position which is deflected from the original position by ΔQ_2, the surface pressure can be expressed as

$$\Delta\gamma = \frac{g\cdot\Delta G_1}{2(t+b)}\left(1+\frac{\Delta Q_2}{\Delta Q_1-\Delta Q_2}\right) = \frac{g\cdot\Delta G}{2(t+b)}\times\frac{\Delta Q_1}{\Delta Q_1-\Delta Q_2} \tag{4}$$

Thus $\Delta\gamma$ can be obtained from (4) by using the data of ΔQ_1 and ΔQ_2. In Fig. 1, the surface pressure—surface area curve of the horse serum albumin is shown, which is obtained by using this apparatus. Curve 1 is at pH = 6, and curve 2 is at pH = 10.

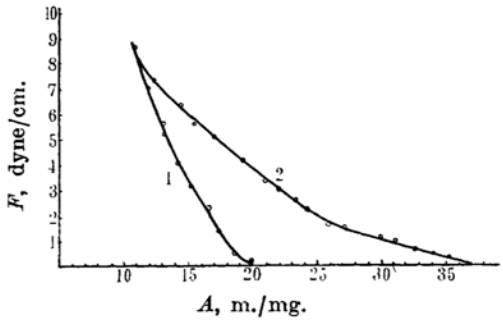

Fig. 1.—F-A curve of horse serum albumin.

Determination of Molecular Weight

The molecular weight of protein can be determined by H. B. Bull's method.[1] In Fig. 2, the film pressure F of the horse serum albumin monolayer multiplied by the film area A per milligram of protein is plotted against the surface pressure F. The experiment has been carried out under pH = 6.

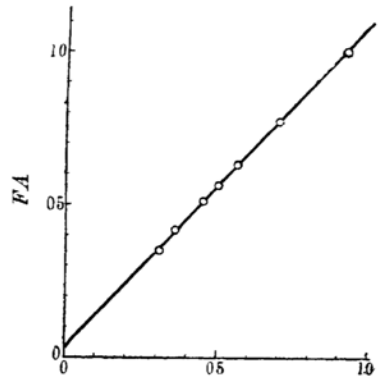

Fig. 2.—Relation between F and FA.

The protein used is the crystalline horse serum albumin which was purified by Dr. Sankichi Nakamura in Chemical Laboratory, Faculty of Science, University of Tokyo, by the sodium sulfate method. Water is distilled several times and is used as the substrate. The surface pressure is measured by the surface balance mentioned above.

Bull has pointed out that $FA-F$ curve should be given as a straight line, which is represented by the following equation:

$$FA = nRT + nS_pF. \tag{5}$$

Here, n is the mol number of the protein on the water surface and S_p is the surface area covered by one mol protein. Accordingly the extrapolated value of FA at zero surface pressure should satisfy the equation,

$$FA = nRT = (m/M)(RT), \qquad (6)$$

where R is the gas constant, T the absolute temperature and m the weight of protein put on the water surface. Thus molecular weight M can be calculated from the above equation. nS_p is equal to the gradient of the curve and can be calculated from Fig. 2. From the points on Fig. 2, values of nRT and nS_p can be calculated by using the method of least squares as follows:

$$nRT = 0.034 \pm 0.006 \text{ and } nS_p = 1.05 \pm 0.11.$$

And therefore, equation (5) can be expressed as

$$FA = 0.034 + 1.05\,F. \qquad (7)$$

And as $T = 19°C.$, the molecular weight of the surface-denatured serum albumin proves to be about $70{,}000 \pm 12{,}000$, which agrees with the data from the measurement of osmotic pressure and others.[6] This tells us that serum albumin does not split into two or more pieces, although it may lose its original shape by surface denaturation.

The surface area covered by 1 mg. of serum albumin is equal to 1.05 m.^{2} This value can be checked by the following compressibility measurement.

The Measurement of the Compressibility of the Film

As expanded film is compressed, it gradually changes into condensed film, and it becomes less and less compressible. At last, at the point where the molecules on the surface are packed most closely, the compressibility comes to its minimum. If the film is further compressed, it will be broken and the compressibility will increase again. The compressibility δ may be defined by

$$\delta = \frac{1}{A}\frac{dA}{dF}. \qquad (8)$$

Where A is the surface area per milligram of protein and F is the surface pressure. Fig. 3 shows the compressibility of the horse serum albumin at various surface areas.

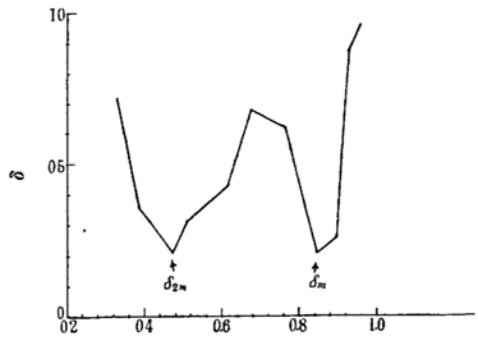

Fig. 3.—Compressibility of horse serum albumin.

As is seen from Fig. 3, the minimum compressibility δ_m has the value of 0.21. The surface pressure F_m and surface area A_m at this point have the value of 12 dyne/cm. and 0.845 m.^{2}/mg. respectively. From the value of A_m the surface area covered by one albumin molecule is calculated to be 9820 Å.^{2}

Fig. 3 shows also that the compressibility has the second minimum δ_{2m}. The surface area at this second minimum point has the value of 0.466 m.^{2}/mg. and this value is equal to about one half of that of A_m. This perhaps shows that at this point, protein has the doubled layer structure.

As is cited above, the surface area of the serum albumin molecule can be calculated from the $FA-F$ curve, and is obtained as 1.05 m.^{2}/mg. The disagreement of this value with that of A_m can be interpreted as follows.

Tonomura[7] has derived, from a statistical mechanical treatment, that if nS_p/A_m has the value of about two, the molecule on the surface is considered to have a round disk shape, while if the ratio has the value near one, it is considered to have an elongated disk shape.

From the present experiment, nS_p/A_m becomes to be equal to 1.24 and this indicates that the serum albumin molecule has an elongated disk shape.

Summary

1. The molecular weight of the surface denatured serum albumin molecule is measured from the $FA-F$ curve. The experiment has shown it to be about 70,000.

2. The surface area covered by one surface-denatured serum albumin molecule is determined to be 9820 Å.^{2} from the compressibility measurement.

(6) H. Neurath, G. R. Cooper, and J. O. Erickson, *J. Biol. Chem.*, **142**, 249 (1942); N. F. Burk, *J. Biol. Chem.* **98**, 353 (1932).

(7) Y. Tonomura, *Kagaku (Science)*, **19**, 565 (1949).

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