The Structure of Surface-Denatured Protein. IV. The Molecular Weight, Surface Area and Shape of the Surface-Denatured Hemoglobin Molecule

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In the previous three papers of this series the author has illustrated the apparatuses for measuring surface pressure and surface potential and by using these apparatuses, the molecular weight, surface area and shape of the surface denatured horse serum albumin have been measured.[1] And in the second paper a theory for the determination of the shape of the protein molecule has been developed.[2]

In this paper the structure of hemoglobin molecule is dealt with. As in the case of horse serum albumin, the molecular weight is first determined by H. B. Bull's method. Then the surface area is determined from the compressibility measurement. And finally the shape of the molecule is determined by the surface diffusion experiment.

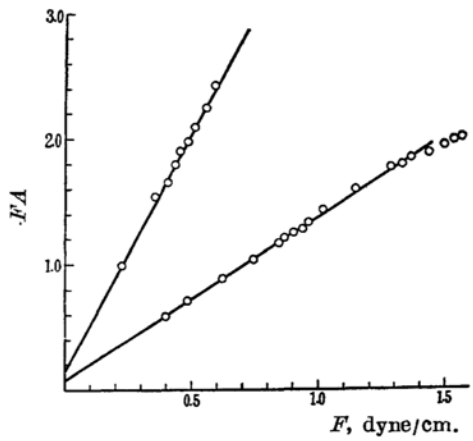

Fig. 1.—FA-F curves at pH=6 and pH=1.8.

Molecular Weight Determination

The method for the determination of molecular weight of surface denatured hemoglobin is just the same as that described in Part I.

Hemoglobin has been purified and crystallized from horse blood by Dr. T. Hosoya of the Institute of Science and Technology, Tokyo University, according to the ethanol method. About 0.2 M KCl solution was used as the substrate, because of the electric measurement purpose. Using the apparatus described in Part I and a thin glass plate, the result was obtained as shown in Fig. 1. The curve of Fig. 1 can be represented by the following equation being obtained by the least squares method:

$$FA=(0.064\pm0.017)+1.32F \qquad (1)$$

Here, F means the surface pressure expressed in dyne/cm. and A means the surface area expressed in m.^{2}/mg. From the equation (10) in Part I, the molecular weight of the surface-denatured hemoglobin is obtained as 35,000± 9,300, which is about a half of that of the native hemoglobin molecule, obtained by sedimentation, diffusion or osmotic pressure measurement.[3],[4] The result is reasonably accounted for by assuming that the hemoglobin molecule splits into two pieces by surface denaturation. This result agrees fairly well with that obtained recently by Michel and Benhamou.[5] Tonomura[6] has calculated the molecular weight of surface denatured hemoglobin to be about 4,000 from the data of Jonix[7] and has concluded that the hemoglobin molecule splits into about sisteen pieces when it is denatured on the surface. However, the measurement of Jonix was carried out under the surface pressure above 1 dyne/cm., and the result obtained by the extrapolation to zero pressure could hardly be expected to be correct. As shown in Fig. 1, the curve deviates from the straight line in the range above 1.5 dyn./cm. Now, if the curve above 1 dyne/cm. is extrapolated to zero pressure the FA value becomes far larger than that of the present experiment.

(1) K. Imahori, This Bulletin, **25**, 7 (1952) hereafter called Part I.
(2) K. Imahori, This Bulletin, **25**, 10 (1952) hereafter called Part II.
(3) N. Gralen, *Biochem J.*, **33**, 1907 (1939).
(4) Svedberg and Pedersen, "Die Ultrazentrifuge," p. 308 (1939).
(5) J. Michael and N. Benhamou, *Compt. rend.*, **228**, 1577 (1949).
(6) Y. Tonomura, *Kagaku*, **19**, 565 (1949).
(7) Jonix, *Biochem. Journ.*, **33**, 1745 (1939).

Guastalla[8] has measured the molecular weight of hemoglobin molecule expanded on the 0.01 *N* HCl solution and obtained the value of 12,000. The present author has measured the molecular weight of hemoglobin expanded on the ammonium sulphate solution of *p*H 1.8. The result is shown in Fig. 1 (curve b). The molecular weight is calculated to be 13,000±2,800. These data may indicate that the molecule splits into four pieces.

Compressibility Measurment

The method for measuring the compressibility of hemoglobin is just the same as that of serum albumin which has been described in Part I. The modified Wilhelmy Balance is used. Hemoglobin used is from the same sample as is used in the previous experiment. Distilled water containing KCl is used as the substrate. As is shown in Fig. 2, the obtained curve has

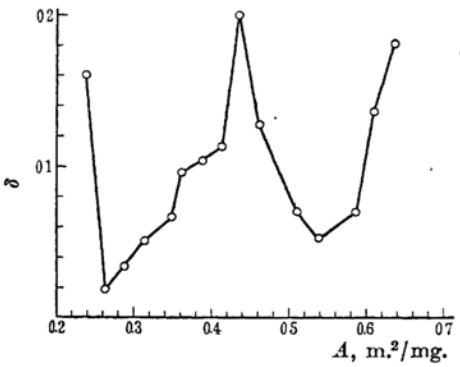

Fig. 2.—Compressibility of hemoglobin Film.

two minima. The one, A_m, is at the surface area of 0.535 m.²/mg. and the other, A_{2m}, is at the surface area of 0.262 m.²/mg. At the first minimum, compressibility, δ_m, is 0.054, and the surface pressure, F_m, is 17.1 dyn./cm., while at the second minimum δ_{2m} is 0.0197, and F_{2m} is 36.6 dyn./cm.

Taking into account that the molecular weight of the surface-dentatured horse hemoglobin is about 35,000 as obtained in the previous experiment, the area per one molecule is estimated te be 3065 Å.² at the first minimum. Davidson and others[9] have reported that horse methemoglobin is like a right-circular cylinder with the diameter of 57 Å. and the height of 34 Å.

The thickness of the hemoglobin film transferred on the metal surface under the surface pressure of about 16 dyn./cm. is measured by the optical method,[10] and found to be about 19 Å. This value is about one half of the height of the methemoglobin molecule described above. The molecular weight of the surface-denatured hemoglobin is about a half of that of the native one. Therefore, it is probable that the horse hemoglobin molecule splits into two pieces and takes the shape just as shown in Fig. 3.

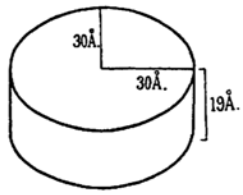

Fig. 3.—The shape of the surface-denatured hemoglobin molecule.

The area of the base of the cylinder-shaped methemoglobin molecule is calculated to be 2560 Å.² from the data by Davidson. On the other hand, even when this cylinder-shaped molecule is packed in one layer compactly on the water surface there is some space around each molecule and the area per one molecule should be about 10% larger than the area of the base of the cylinder, namely, 2816 Å.² per one molecule.

The area at the first minimum is obtained to be 3065 Å.² and this fact shows that the surface-denatured hemoglobin molecule at this point is in the state of closest packing.

If the film were compressed still more it would collapse, or two denatured homoglobin molecules would begin to join into the one original cylinder-shaped molecule. Taking into account that the A_{2m} is about one half of A_m, the film is considered to be doubled at the second minimum. Because, at A_{2m} one molecule covers about 1490 Å.² or two molecules cover about 2980 Å.², which is nearly equal to the above calculated value 2816 Å².

Moreover, δ_{2m} is somewhat smaller than one half of δ_m—the film at δ_{2m} is more than twice as incompressible as that at δ_m.

The Shape of the Surface-Denatured Horse Hemoglobin Molecule

From the result of the previous experiment it is supposed that the surface denatured hemoglobin molecule would have the shape as shown in Fig. 3.

The shape can be determined from the

(8) Guastalla, *Compt.* rend., **208**, 1078 (1939).
(9) J. Bones-Watson, E. Davidson and M. F. Perutz, *Proc. Roy. Soc.*, **191A**, 83 (1947).
(10) K. Imahori and Y. Yoneyama, unpublished.

measurement of the diffusion constant on the surface. The theoretical treatment, on which the present experiment has its foundation, has been dealt with in Part II of this series.

The method for measuring the surface diffusion constant is just the same as that used in the case of serum albumin. Distilled water containing KCl is used as the substrate and castor oil is used as the balancing substances in the present case. The surface pressure is about 0.8 dyne/cm.

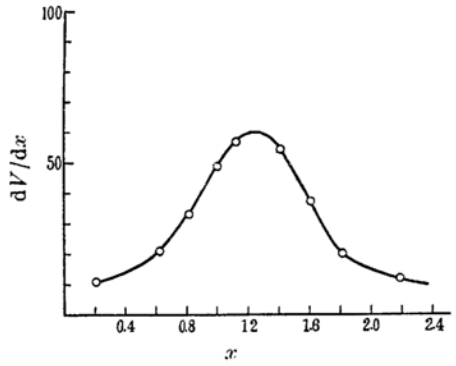

Fig. 4.—Concentration gradients after 7.2 hours of diffusion.

The result is shown in Fig. 4, which shows the gradients of surface potential at various points after 7.2 hours diffusion. In the figure, V means the surface potential expressed in Volt, and x means the distance expressed in cm. From this curve the diffusion constant is calculated, by the inflection point method, to be 2.78×10^{-6} cm.^{2}/sec. Using this value, and also other data such as $V=0.749$[4], $d=$ 19 Å. and $M=35.000$, f/f_0 is calculated from the equation (11) of part II to be 1.003. And thus c/a can be obtained from the Fig. 1 of Part II to be 1.12.

From this result it would seem that the surface-denatured hemoglobin molecule is an almost perfectly circular disk. In conclusion, the hemoglobin molecule is considered to split into two equal disks, the radius and the height of which are calculated to be about 30 Å. and about 19 Å., respectively, when it is denatured on the surface of the distilled water.

Summary

1. The molecular weight of the surface-denatured hemoglobin is calculated from the FA-F curve, to be about 35,000, which shows that the hemoglobin molecule splits into two pieces by surface denaturation at pH$=6$.

2. From the compressibility data, and surface denatured hemglobin molecule is considered to cover about 3065 Å.^{2} of area.

3. The ratio of two axes is obtained by the surface diffusion experiment to be 1.12.

4. The thickness of the molecule is determined to be 19 Å. by the optical method, and the surface-denatured hemoglobin molecule is considered to be a perfectly circular disk, the radius of which is about 30 Å. and the thickness about 19 Å.

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