The Structure of Surface-Denatured Protein. II. Relation between the Surface Diffusion Constant and the Shape of the Protein Molecule (Theoretical)

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In the previous paper of this series(1), the molecular weight of the surface-denatured serum albumin has been obtained: 70,000 from the $FA-F$ curve. The surface area of the serum albumin is also obtained: 9820 Å.[2] from the compressibility measurement. Now it is desired to clarify the shape of the surface-denatured protein.

The shape of the native protein molecule has already been investigated by the measurement of the viscosity of the solution, determination of its diffusion constant, measurement of the sedimentation equilibrium under the ultracentrifugal force, etc.

The shape of the surface-denatured protein is likely to be determined by the analogous experiment. Of those methods the measurement of the viscosity and the diffusion constant are applicable for the surface film.

In our laboratory the latter method has been carried on. In this paper the relation between the surface diffusion constant and the shape of the molecule will be derived theoretically.

Relation between the Diffusion Constant and the Friction Ratio

It is perhaps quite natural to postulate that the surface-denatured protein molecule has the form of an elliptic disk, which floats upon the water surface. Suppose at first the disk is circular.

If the relative speed of the disk and water be taken as U, then the frictional force K can be given as

$$K=6\pi\eta p'U \tag{1}$$

where, η is the viscosity coefficient of water. According to Lamb(2), p' is expressed as $8r/9\pi$, r being the radius of the disk. The driving force acting on the protein molecules which are contained in 1 cm.[2] of area can be expressed as $-\mathrm{d}F/\mathrm{d}x$, where F is the surface pressure of protein film. One square centimeter of area containe $(\rho/M)\times N$ protein molecules, in which ρ is the mass of protein contained in 1 cm.[2] of area, M is the molecular weight of protein, and N is the Avogadro's number. Thus, the driving force acting on one protein molecule can be shown as $-(M/\rho N)(\mathrm{d}F/\mathrm{d}x)$. This force must be equal to K in magnitude and must have the opposite sign to that of K. When surface film is gaseous we can put

$$F=\frac{R}{M}\rho T \tag{1'}$$

so that $\frac{\mathrm{d}F}{\mathrm{d}x}=\frac{R\cdot T}{M}\cdot\frac{\mathrm{d}\rho}{\mathrm{d}x}$ must hold, and finally we get

$$6\pi\eta p'U=\frac{RT}{\rho N}\frac{\mathrm{d}\rho}{\mathrm{d}x} \tag{2}$$

or

$$U=\frac{R\cdot T}{6\pi\eta\rho Np'}\frac{\mathrm{d}\rho}{\mathrm{d}x} \tag{2'}$$

The mass of protein which diffuses in one second across a line of 1 cm. long will be expressed as $U\rho$. On the other hand from Eq.

(1) K. Imahori, This Bulletin, **25**, 7 (1952).

(2) H. Lamb, "Hydrodynamics," 6th edition, Cambridge, 1932, p. 605.

(2) this can be expressed as follows:

$$U\rho = -\frac{RT}{6\pi\eta Np'}\frac{d\rho}{dx} \tag{4}$$

As mentioned above, $p'=8r/9\pi$, and the Eq. (4) may be expressed:

$$U\rho = -\frac{3RT}{16\eta Nr}\frac{d\rho}{dx} \tag{4'}$$

The diffusion constant D_0 can be defined as

$$U\rho = -D_0\frac{d\rho}{dx} \tag{5}$$

so that from Eqs. (4') and (5) the following relation will be obtained:

$$D_0 = \frac{3RT}{16\eta}\frac{1}{N\cdot r} \tag{6}$$

The suffix 0 means that the molecule is circular.

As $16\eta r/3$ means the frictional force acting on the circular disk when it is floating at a unit speed, we can put

$$\frac{16\eta r}{3} = f_0$$

Then (6) reduces to:

$$D_0 = \frac{R\cdot T}{N}\cdot\frac{1}{f_0} \tag{7}$$

When the disk is not circular, but elliptic, the frictional force may be expressed as f, and just like for the circular disk, the next relation can be obtained for the elliptic disk:

$$D = \frac{R\cdot T}{N}\frac{1}{f} \tag{8}$$

From (7) and (8) is obtained:

$$\frac{D}{D_0} = \frac{f_0}{f} \tag{9}$$

Now, the molecular weight M of a circular disk-shaped protein molecule can be expressed by r (the radius of disk), d (its thickness), V (the reciprocal of the density) and N (Avogadro's number), as follows

$$M = \pi r^2 d\frac{N}{V} \tag{10}$$

Combining with (6) and (9), (10) can be expressed as

$$M = \frac{\pi dN}{V}\cdot\left(\frac{3}{16\eta}\right)^2\cdot\left(\frac{RT}{ND_0}\right)^2 = \frac{9\pi dR^2T^2}{256\,\eta^2ND^2V}\left(\frac{f_0}{f}\right)^2 \tag{11}$$

This shows the relation between the diffusion constant D and the friction ratio f/f_0. In the equation, M can be determined by the method described in the previous paper[1]. The thickness d can be determined by the optical method which will be reported in the later paper[3]. And thus f/f_0 can be determined from equation (11), if the value of D is known. The method for determination of the value of D will be reported in part 3 of this series.

Relation between the Friction Ratio f/f_0, and the Ratio of Two Axes of Ellipse c/a

The friction ratio can be determined from the above equation (11). But in order to determine the actual shape of the ellipse, the relation between f/f_0 and c/a must be derived. In this section, this relation has been derived as follows.

The frictional force acting on the disk which floats on the water surface is supposed to be just a half of the force acting on the disk which moves through the water in its edgeways direction. And when the elliptic disk flows on the water surface, the frictional force acting upon the disk is expressed by

$$K = 6\pi\eta RU$$

where U is the relative velocity of the disk against water, η is the viscosity of water and R is the function of the magnitudes of two axes of the ellipse.

According to Lamb[2], for the movement in its a-axis direction, R can be expressed as R_a and is given by the relation:

$$R_a = \frac{4}{3}\frac{ac}{\chi_0+\alpha_0a^2} \tag{13}$$

and

$$\chi_0 = ac\int_0^\infty\frac{d\lambda}{\sqrt{(a^2+\lambda)(c^2+\lambda)\lambda}} \tag{14}$$

$$\alpha_0 = ac\int_0^\infty\frac{d\lambda}{\sqrt{(a^2+\lambda)^3(c^2+\lambda)\lambda}} \tag{15}$$

The former integration can be carried out by setting $\lambda = t^2$

(3) K. Imahori and Y. Yoneyama, unpublished.

$$\int_0^\infty \frac{d\lambda}{\sqrt{(a^2+\lambda)(c^2+\lambda)\lambda}} = \int_0^\infty \frac{1}{t\sqrt{(a^2+t^2)(c^2+t^2)}} \cdot \frac{d\lambda}{dt} \cdot dt = \int_0^\infty \frac{2dt}{\sqrt{(a^2+t^2)(c^2+t^2)}} = \frac{2}{c}K(e) \qquad (16)$$

$K(e)$ means the complete elliptic integral of the first order and e is the eccentricity of the ellipse as given by the following relation:

$$e^2 = \frac{c^2-a^2}{c^2}$$

The integration of α_0 can be carried out as follows. As in the case of χ_0, $\lambda = t^2$ is put in (14)

$$\alpha_0 = \int_0^\infty \frac{d\lambda}{\sqrt{(a^2+\lambda)^3(c^2+\lambda)\lambda}} = \int_0^\infty \frac{2dt}{\sqrt{(a^2+t^2)^3(c^2+t^2)}} = \frac{2}{c^2-a^2}\left[\int_0^\infty \sqrt{\frac{(c^2+t^2)}{(a^2+t^2)}}\,dt - \int_0^\infty \frac{dt}{\sqrt{(a^2+t^2)(c^2+t^2)}}\right]$$

The second term in the bracket is just equal to the integration of Eq. (15) and can be given as the complete elliptic integration of the first kind. The former term is the one of the modification of the complete elliptic integral of the second kind. And thus α_0 can be expressed as follows:

$$\alpha_0 = \frac{2}{c^2-a^2}\left[\frac{c}{a^2}\cdot E(e) - \frac{1}{c}\cdot K(e)\right] = \frac{2}{a^2c(c^2-a^2)}[c^2E(e)-a^2K(e)] \qquad (17)$$

Putting (16) and (17) into (13), the following will be obtained

$$R_a = \frac{4}{3}\frac{1}{\frac{2}{c}\cdot K(e) + \frac{2}{c(c^2-a^2)}[c^2E(e)-a^2K(e)]} = \frac{2}{3}\frac{c}{K(e)+\frac{1}{c^2-a^2}[c^2E(e)-a^2K(e)]} \qquad (18)$$

And the frictional force acting on the disk can be given from equation (12) as:

$$K_a = \frac{4\pi\eta Uc}{K(e) = \frac{1}{c^2-a^2}[c^2E(e)-a^2K(e)]} \qquad (19)$$

In the similar way for the movement in b-axis direction the frictional force K_b can be given as:

$$K_b = \frac{4\pi\eta Uc}{K(e)+\frac{1}{e^2}[K(e)-E(e)]} \qquad (20)$$

The molecule on the surface moves quite at random, so frictional force must be averaged statistically for all directions. This can be done approximately by the following relation:

$$\frac{1}{K} = \frac{1}{2K_a} + \frac{1}{2K_b} \qquad (21)$$

By putting (19) and (20) into (21), K can be expressed as

$$K = \frac{8\pi\eta Uc}{3K(e)} \qquad (22)$$

while, as mentioned above, f_0 can be defined by

$$f_0 = \frac{16\eta r}{3} \qquad (23)$$

Now, similarly f can be defined by

$$f = \frac{8\pi\eta c}{3K(e)} \qquad (24)$$

This defininition is quite logical, because if the relation $c=a=r$ holds, f reduces to f_0, or when the disk is circular f becomes just equal to f_0.

From (23) and (24) and also by using the relation of

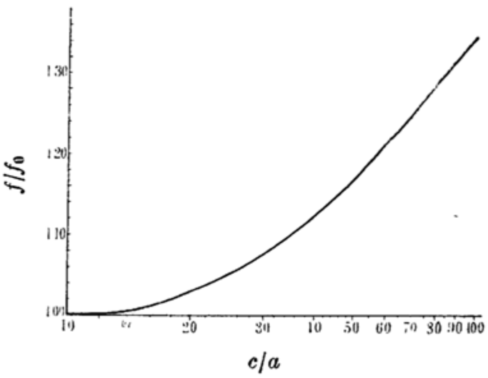

Fig. 1.—Relation between f/f_0 and c/a.

$$ac=r^2$$

the friction ratio is expressed as follows:

$$\frac{f}{f_0}=\frac{\pi}{2K(e)}\cdot\frac{c}{r}=\frac{\pi}{2K(e)}\sqrt{\frac{c}{a}}$$
$$=\frac{\pi}{2K\left(\sqrt{\frac{c^2-a^2}{c^2}}\right)}\cdot\sqrt{\frac{c}{a}} \qquad (25)$$

This shows the relation between f/f_0 and c/a. This relation can be expressed as Fig. 1, and the value of c/a can be derived when the value of f/f_0 is found by the method mentioned above.

Summary

1. The relation between the friction ratio and the surface-diffusion constant has been derived.

2. The relation between the friction ratio and the ratio of two axes of an ellipse has been derived.

3. The relation between f/f_0 and c/a has been shown in a curve which makes the estimation of c/a very simple.

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