The Structure of Surface-Denatured Protein. VI. The Effect of pH of Substrate upon the Limiting Area of Protein Monolayer

By Kazutomo IMAHORI

(Received November 26, 1953)

Introduction

Gorter[1] has investigated the influence of *p*H of the substrate upon the spreading of various proteins, by measuring the spreading areas after the protein has been in contact with solutions of different *p*H for a period of one or two minutes. He observed that such protein as egg albumin or serum albumin, spread well at the *p*H of its isoelectric point, as well as in the case of strong alkaline and strong acid solutions, but on both sides of the isoelectric point, little spreading could be obtained. But the low spreading disappeared, when enough salt was added to the substrate. These results can be explained[2] by assuming that at *p*H values, where the effective protein charge is high, the spreading tendency is at a minimum. Yet, the truth of the above assumption has not been ascertained. The author has studied the size and the shape of protein molecules expanded on substrates of various *p*H values, and has considered the cause of spreading from these results.

1) E. Gorter, H. Ormondt, and F. Dom, *Proc. Kon. Akad. v. Wet.*, 35, 838 (1933).
2) E. Gorter 'Surface tension and films' Schmit's Chem. of the amino acids and proteins. p. 428.

The Limiting Area

The limiting areas of the surface films of egg albumin as well as horse serum albumin have been measured. Both samples are in the pure crystalline state, being prepared by Drs. S. Nakamura and H. Sugano at the Chem. Inst. of Tokyo University. The apparatuses used for this measurement are a surface balance and than ellipsometer, the former of which has been described in the first report of this series[3], while the details of the latter will be published shortly. Three buffer solutions having the *p*H values of 1, 3, (HCl buffer) and 5 (acetate buffer), are used as the substrates.

The F—A relations of serum albumin films on these three buffers are shown in Fig. 1. These would suggest many things. First of all, the curve on *p*H=5 is of a condensed type, while the other two are of an expanded type. The curve of *p*H=1 breaks at the area of 0.845 m^2/mg, and the curve of *p*H=5, at 0.76 m^2/mg, but the curve of *p*H=3, at the area of 0.637 m^2/mg.

Similar features can be seen in the case of egg albumin. In Fig. 2, δ—A relations of egg albumin

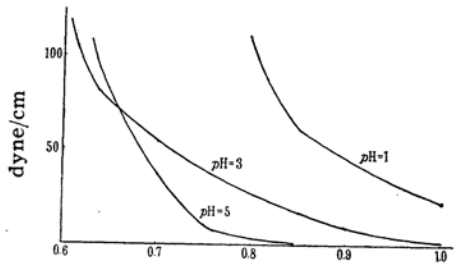

Fig. 1.

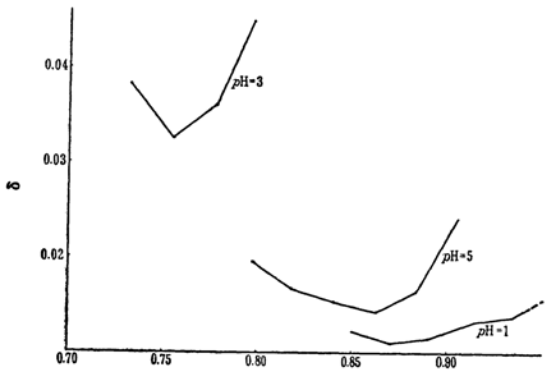

Fig. 2

are shown instead of those of F—A, where δ means the compressibility, being defined by the following relation:

$$\delta = -\frac{1}{A} \cdot \frac{dA}{dF}$$

The δ—A relations on pH=1 and pH=5 are similar, and the minima of both curves are at 0.87 and 0.86 m²/mg respectively. But the curve of pH=3 has a far smaller limiting area (0.755 m²/mg). Fig. 2 shows also that the δ min (the value of δ at the minimum point) on pH=3 is greater than those on pH=1 and pH=5. This means of course, that the film of egg albumin which expanded on the substrate of pH=3 is more compressible than those on pH=1 and pH=5. This fact seems to have some relation to the results of the following experiment.

The films which expanded on the substrates of various pH values were transferred on the metallic slide, by Blodgett's method, under the surface pressure of 13 dynes/cm, and 30 dynes/cm.

The thicknesses of these films were measured by using the ellipsometer. The results were as follows. The films expanded on pH=1 and pH=5 showed the same and constant thickness,—about 10 Å—if they were built up under the pressure of 13 dynes/cm or 30 dynes/cm. But the film on pH=3 had as well the thickness of 10 Å when it was built up under the pressure of 13 dynes/cm., but its thickness increased to 25 Å, when it was built up under the pressure of 30 dynes/cm. This result would suggest that the egg albumin molecules, which were denatured and expanded on the solution of pH=3, might be easily again folded into thicker ones, being in accordance with the fact of the large δ value of this film.

The Size and the Shape

The molecular weight of egg albumin which expanded on the solutions of pH=1 and pH=3, has been determined by the method of Bull and Guastalla[4]. The molecular weight thus obtained was about 42,000 in both cases. This value is just between those obtained by Bull and Guastalla. On the other hand, the molecular weight of serum albumin has been obtained as 70,000 in the previous paper[3].

From these values and the limiting areas obtained above, we can calculate the surface area of each protein molecule as shown on the third line of Table I.

The shape of a protein on each substrate might be determined by the experiment of surface diffusion. The details of the experiment and also of the way of calculation, were given in the second and third reports of this series[5,6].

TABLE I

	Egg albumin			Serum albumin		
pH	1	3	5	1	3	5
surface area per molecule	6065 Å²	5260 Å²	60000 Å²	9830 Å²	7380 Å²	8840 Å²
axial ratio	30:1	15:1		20:1	10:1	8:1
short axis	28.5 Å	37.5 Å		44.4 Å	54.5 Å	66.7 Å
long axis	855 Å	562 Å		888 Å	545 Å	533 Å

The results are also shown on the fourth line of Table I. The shape, being assumed to be an elliptic disk, is expressed in terms of the axial ratio. On the fifth line of the table, the lengths of two axes are given.

The data in Table I suggest that the shape of the snrface-denatured protein molecule of both albumin species depends on the pH value of the substrate. The molecules expanded on the acid substrate have extremely elongated shapes. This conclusion ascertained by experiment of surface viscosity. Fig. 3 shows the results of the mea-

3) K. Imahori, This Bulletin, **27**, 7 (1952).
4) H. B. Bull, "Advances in Protein Chemistry." Vol. 3.
5) K. Imahori, This Bulletin **27**, 9, (1952).
6) K. Imahori, This Bulletin **27**, 11 (1952).

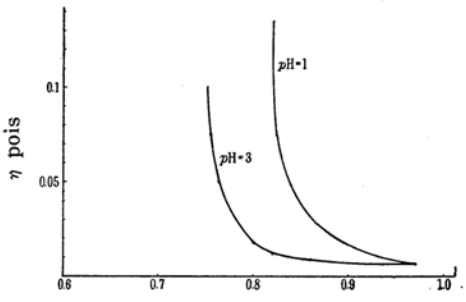

Fig. 3

surements of surface viscosity against the surface area, indicating that the film of egg albumin expanded on pH=1 solution has far greater viscosity than that on pH=3. Similar results are obtained, if we measure the surface viscosity of the serum albumin on pH=1 and pH=5. The high viscosity of both albumins on pH=1, shows that these protein molecules have elongated shapes on the acid substrate.

Discussions

The results of the preceding experiments show that each molecule of two albumin species takes a different form in the three different solutions. The molecule expanded on the solution of pH=1, has the most elongated shape. It forms well expanded, quite thin, and the least compressible rigid films.

The molecule expanded on pH=3, shows a less elongated shape. As it is more compressible than that on pH=1, it is presumably folded into the thicker film under high surface pressure.

The molecule on pH=5, shows also a less elongated shape as in the case of pH=3. But it makes a well-expanded, incompressible rigid film.

The F—A curve of this latter film is of the condensed type, indicating that interaction between molecules is fairly small.

These differences may be explained to some extent as follows. On the solution of pH=1, the basic residues of the albumin molecule, such as arginine, or lysine are ionized and carry the positive charge, while the acid residues such as glutamic acid or asparatic acid are not ionized in this pH range and consequently carry no charge.

The positive charges of those basic residues act repulsively on each other. On the other hand, they are very hydrophilic and are apt to put themselves into the water. The former causes the albumin molecules to be perfectly unfolded, and to take an extremely elongated shape. The latter causes them to lie flat on the water surface, and to make a stable rigid film. Each molecule carries a number of net positive charges, which cause the molecules to repulse each other, and makes the F—A curve into an expanded one.

On the substrate of pH=5, on the other hand, most of the acid and basic residues are ionized, and the albumin molecule contains about an equal number of negative and positive charges upon it. These charges of different signs, acting attraction force on each other and leading to inter- and intramolecular salt-like bonds, as well as hydrogen bonds, and S-S bonds, will prevent the albumin molecule from being perfectly unfolded. Thus, the albumin molecule on pH=5, containing bended structure, takes rather a round shape. The intra-molecular bonds also make the polypeptide chain assume a definite structure, which leads to the comparatively large limiting area and low compressibility as is seen in the above experiment. As the molecule contains many ionized and hydrophilic residues, it can make the stable, incompressible and rigid film. On the substrates of pH=3, the number of positively charged residues of the albumin molecule is less than that of pH=1, while, some of the acid residues can be ionized in this case. Thus, the intra-molecular repulsive force is not so strong as that of pH=1, while the intramolecular bonds are not enough to hold the rigid and bended structure, on the water surface. Thus, the molecule on pH=3 is very flexible, yields easily to external force and causes the low limiting area. A small number of charges make the film somewhat hydrophobic and compressible into a thicker film, under high surface pressures.

This contraction and expansion of the film caused by the variation of pH of the substrate was observed with a mixed film of octadecyl amine and stearic acid as well.

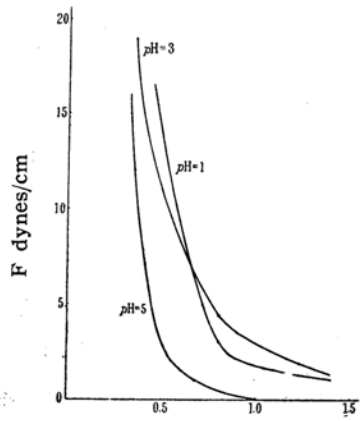

Fig. 4

The details of the experiment will be published elsewhere in the near future.

The following experiment supports our explanation. In Fig. 4, the F—A curve of the heat denatured serum albumin is shown. The procedure was nearly the same as that of S. Staelberg[7]. The same crystalline serum albumin was dissolved in 60% n-propyl alcohol containing 0.5 M sodium acetate, and denatured by heating at 100°C for 30 minutes. The protein spread from this solution gave the limiting areas of 0.82 and 0.55 m^2./mg. on the substrates of pH=1 pH=5 respectively. The F—A curve of the film on pH=3 broke slightly at the area of about 0.82 m^2./mg., but the film might be compressed to the limiting area of 0.55 m^2./mg. The small limiting area on pH=5 means that the intra-molecular bonds were broken by heat, and so the molecules were readily compressible by the attraction between residues of different signs. On the substrate of pH=1, however, few differences were found between the heat-denatured and not heat-denatured proteins. The breaking of intra-molecular bonds by heat resulted in a good expansion of protein on the substrate of pH=3. As the repulsive force was not so remarkable as on pH=1, the molecule could be easily compressed into the compact state as on pH=5.

7) Stina Staelberg, *Trans. Faraday Soc.*, **35**, 1416 (1939).

Conclusion

(1) The size and shape of the egg and serum albumins have been measured on three buffer solutions having the pH values of 1, 3, and 5 respectively.

(2) The film on pH=1 is a well-expanded, incompressible and rigid one. That on pH=5 is as well expanded, but the film on pH=3 is of a not well expanded but easily compressible type.

(3) These differences have been discussed from the points of charges and intra-molecular bonds.

The author wishes to express his hearty thanks to Prof. Bun-ichi Tamamushi, for his kind inspection of this paper. Some part of the present work has been aided by Miss Mitsuko Hachiwaka and Miss Harumi Matsui, to whom the author's thanks are due. This experiment is aided by the Scientific Research Encouragement Grant from the Ministry of Education.

Chemical Iustitute, College of General Education, Tokyo University, Tokyo