Surface Films of Physically and Chemically Treated Ovalbumin (Dynamic Studies on Monolayer. II)

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Studies have previously been made of monolayer properties of crystalline ovalbumin in which it has been shown that the monolayer structure can be examined conveniently in its spreading process and this spreading of monolayer at the air-water interface was found to be directly linked with the denaturation[1]. The structural status of the spread monolayer can now be investigated to a certain extent from the measurements of surface pressure or potential changes with time at a constant area of a given substrate.

It appears that relatively few studies have been made on the measurement of surface pressure changes with time. Although important roles of experimental conditions, that of substrate in particular, have been fully emphasized, it must also be pertinent to investigate the effect of physical or chemical treatments on protein prior to spreading. Proteins are known to be denatured both by chemical and physical treatments. The surface denaturation of proteins was studied by Bull and Neurath[2-4]. Kaplan also has extensively studied the effect of physical and chemical treatments on ovalbumin monolayers[5-8].

Since denaturation is essentially an intramolecular phenomenon, involving changes in an unknown structure, it can be investigated and described in terms of these changes, whether physical, chemical or biological. Among others, the effect of heat with infrared irradiation, acid and alkali treatments on the ovalbumin molecule before spreading are investigated and discussed here.

Experimental

Apparatus.—The surface balance used is the same as the one previously described[1]. Wilhelmy's vertical plate type is found to be more convenient than Langmuir and Adam's apparatus for the measurement of pressure changes with time.

Materials.—Ovalbumin was obtained by the usual method as described before[1]. This ovalbumin is treated with heat, acid and alkali, respectively. Except for the treatment with heat, acid and alkali treatments are based partially on MacPherson and Heidelberger's studies[9]. The temperature condition of heat treatment in this case is made somewhat mild. The protein under those conditions is expected to be slightly denatured as indicated by the nitroprusside test[10]. This test was also applied to the untreated native protein with negative result. Conditions adopted hereby are summarized in Table I.

Experimental procedure.—Protein is spread from its aqueous solution by means of a micropipet over a large area (2.5 m^2/mg.) and the film is kept there for exactly 5 minutes before the start of compression for *F-t* curve measurement. To determine pressure-area diagrams, the time allowed for after spreading is 20 minutes instead of 5 minutes. The most commonly used substrate is aqueous 20% ammonium sulfate solution at pH 4.8 since this is one of the best substrates[1].

TABLE I
CONDITIONS OF OVALBUMIN TREATMENTS

Modification	Concn.	Reagent used	Temp.	Time	pH	Nitroprusside test
Acid	0.224 mg./cc.	0.1 N HCl	2~4°C	17 hrs.	2.2	+
Alkali	〃	0.04 N NaOH	2~4°C	17 hrs.	9.6	+
Heat	〃	infrared light 100 V. 250 W	42°C	1/3 hrs.	—	+

1) M. Kashiwagi, This Bulletin, **31**, 176 (1958).
2) H. B. Bull and H. Neurath, *J. Biol. Chem.*, **118**, 163 (1937).
3) H. B. Bull, ibid., **125**, 585 (1938).
4) H. Neurath, *J. Phys. Chem.*, **40**, 361 (1936).
5) J. G. Kaplan, *J. Colloid Sci.*, **7**, 382 (1952).
6) J. G. Kaplan and M. J. Fraser, *Biochim et Biophys. Acta*, **9**, 585 (1952).
7) J. G. Kaplan, D. H. Andrews and M. J. Fraser, *J. Colloid Sci.*, **9**, 203 (1954).
8) J. G. Kaplan and M. J. Fraser, *J. Biol. Chem.*, **210**, 57 (1954).
9) C. F. C. MacPherson and M. Heiderberger, *J. Am. Chem. Soc.*, **67**, 574 (1945).
10) D. Glick, "Methods of Biochemical Analysis", Vol. 1, p. 20 (1954).

Results

Fig. 1 represents measured surface pressure changes ΔF with time of heat-treated ovalbumin monolayers at various surface areas and on the substrate of 0.1 N hydrochloric acid. Pressure-time curves of the same sample on the substrate of 20% ammonium sulfate solution are also shown in Fig. 2. It can be seen from Fig. 1 that F increases with time, but the rate of increase is markedly dependent on surface area, being only slightly noticeable at 1.18 m²/mg., while it is very pronounced at 0.914 m²/mg. On the other hand, as shown in Fig. 2, F decreases in the initial stage on ammonium sulfate substrate and then becomes constant at 0.92 m²/mg., while at larger areas, F seems to increase very slowly but linearly with time. Surface pressures of acid-treated ovalbumin remain practically constant for the whole period of the experiment, or very slightly increase on the substrate of 20% ammonium sulfate (Fig. 3). F-t

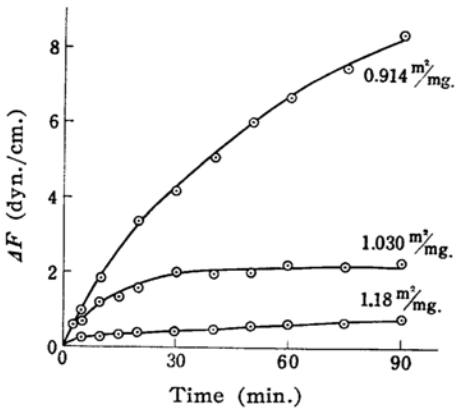

Fig. 1. Surface pressure changes of heat-denatured ovalbumin (Substrate 0.1 N HCl, 14°C).

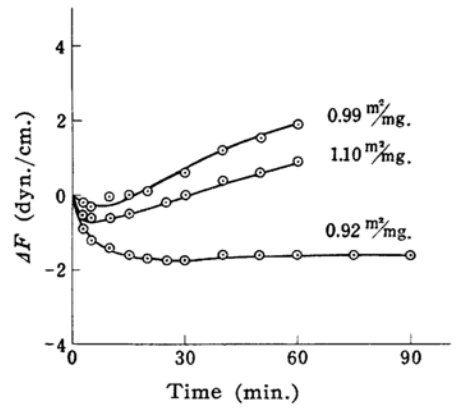

Fig. 2. Surface pressure changes of heat-denatured ovalbumin (Substrate 20% $(NH_4)_2SO_4$, 15°C) pH 4.8.

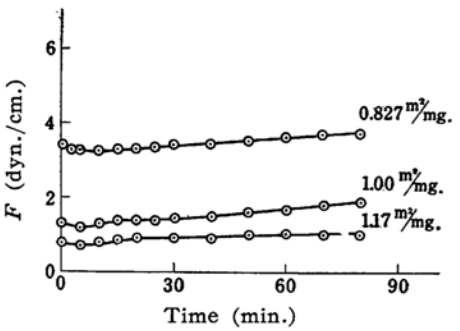

Fig. 3. Surface pressure changes of acid-treated ovalbumin (Substrate 20% $(NH_4)_2SO_4$, pH adjusted 7.1, 19°C).

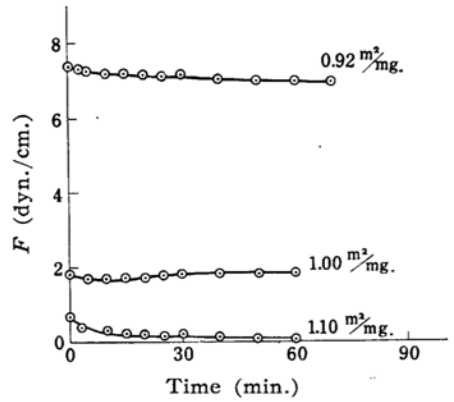

Fig. 4. Surface pressure change of alkali-treated ovalbumin (Substrate 20% $(NH_4)_2SO_4$, 16°C) pH 4.8.

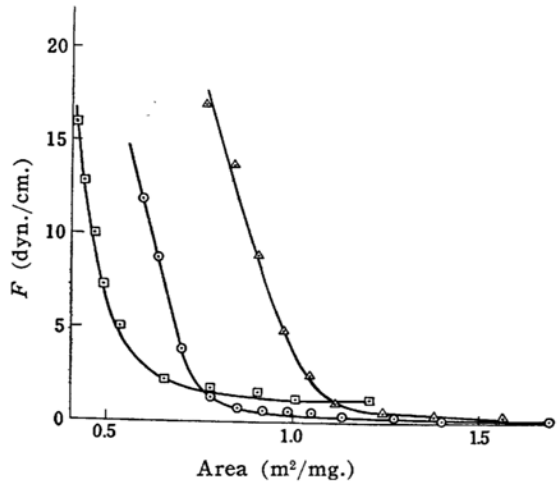

Fig. 5. Pressure-area diagram of heat-treated ovalbumin.
△ 20% $(NH_4)_2SO_4$ Substrate pH 4.8
⊙ 0.1 N HCl Substrate
⊡ untreated ovalbumin on 0.1 N HCl

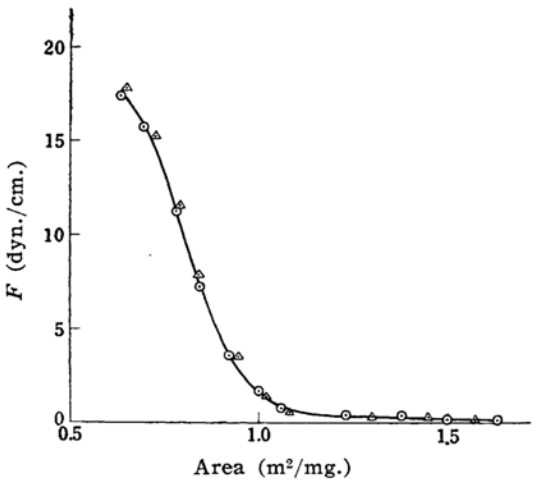

Fig. 6. Pressure-area diagrams of ovalbumin (Substrate 20% $(NH_4)_2SO_4$).
△ alkali-treated
⊙ acid-treated
PH of Substrate
△ 4.8
⊙ 7.1

curves of alkali-treated ovalbumin soon reach a final equlibrium pressure and remain constant (Fig. 4).

All the actual measurements of pressure changes with time are carried out every three minutes at least, but the recording of all points on *F-t* curves are not attempted for convenience' sake. Pressure-area diagrams of these treated ovalbumin monolayers are shown in Figs. 5 and 6, respectively, together with that of untreated native ovalbumin for comparison. *F-A* curves of both acid- and alkali-treated protein are identical within the limit of the present experimental condition, as indicated in Fig. 6.

Discussion

It may be expected that all these treatments applied here would not cause substantial structural change in the protein[9,11]. The present condition of heat treatment was rather milder than in the case of ordinary conditions for denaturation, but it is interesting to note that surface pressure of heat treated ovalbumin increases with time on 0.1 N hydrochloric acid as indicated in Fig. 1, while with the untreated native protein surface pressure decreased with time on the same substrate[1], indicating partial desorption. Pressure-area diagrams of the former also shift obviously from that of the untreated. By heat treatment the intramolecular cohesion forces are lessened to such an extent that the protein spreads to a larger area than they normally occupy[2,8] and the ease of molecular unfolding increased by heat must have retarded the factor to cause desorption from the surface.

The protein denaturation is often followed inevitably by intermolecular aggregation, which makes the interpretation of the denaturation effect very difficult, but ovalbumin molecules under the present condition would not proceed progressively to marked aggregation or dissociation[9,11].

We have seen in Figs. 3 and 4, that under a constant film area, the surface pressure is independent of time, that is

$$\left(\frac{\partial F}{\partial t}\right)_{A_0} = 0$$

where A_0 refers to co-area.

But since we have a general relation

$$\left(\frac{\partial A_0}{\partial t}\right)_F = -\left(\frac{\partial F}{\partial t_0}\right)_{A_0} \Big/ \left(\frac{\partial F}{\partial A_0}\right)_t$$

where $\left(\frac{\partial F}{\partial A_0}\right)_t$ shows a finite value, $\left(\frac{\partial A_0}{\partial t}\right)_F$ vanishes, which means, that A_0 is independent of time. Therefore we can expect that under such a condition, molecules in the film would be fully denatured in accordance with our former report[1].

Summary

Monolayer properties of acid, alkali and heat-treated ovalbumin are investigated from the measurements of surface pressure changes with time and surface pressure-area diagrams.

These treated specimens are found to be characteristically different from the untreated native protein in their surface behavior. The process of surface denaturation is also discussed.

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11) V. A. Belister and T. V. Saenko, *Biokhimiya*, 22, 274 (1957).