SURFACE GELATION OF OVALBUMIN MONOLAYERS AT THE AIR-WATER INTERFACE

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INTRODUCTION

It has long been known that protein monolayers go into the gel state when compressed to a certain area[1]. However, only slight attention has been paid to this gelation phenomenon[2]. In a previous paper[3] we reported that such a gelation occurs at an unexpectedly large area with ovalbumin monolayers at the air-water interface More recently we have drawn attention to the fact that the largest area at which the protein film exhibits viscoelasticity, *i.e.* the critical gelation area, is, though taken as characteristic for a given protein, greatly dependent upon both the spreading solution and the subsolution. This paper deals primarily with some new information about the film state of ovalbumin revealed from the measurement of the critical gelation area.

EXPERIMENTAL

Monolayers of ovalbumin from a 0.01 % aqueous ovalbumin solution were spread on water in a Langmuir trough, with the initial surface concentration below 0.2 mg/m^2. Simultaneously with the successive compression of the films, their viscoelasticity was detected by employing the surface rheometer described elsewhere by one of us (K.I.)[4], and the critical area at which viscosity or viscoelasticity begins to develop was recorded. As reported in a previous paper[3], the rheological behavior of gelled film was investigated by analysing the deformation-time curve under a certain constant stress and representing its result by a corresponding mechanical model. The film subjected to a certain stress may exhibit instantaneous elasticity, delayed elasticity, or simple viscosity, depending upon the film state. The critical area at which these mechanical responses against stress begin to be exhibited, was respectively designated as A_i for instantaneous elasticity, A_d for delayed elasticity and A_v for simple viscosity.

The sample of crystalline ovalbumin used was prepared from fresh hen eggs by the sodium sulfate method[5]. The composition of subsolution will be described for each particular case.

RESULTS AND DISCUSSION

1. *Subsolution*

The effect of pH of subsolution on the critical gelation area was first investigated. In the pH range 2$\sim$8, secondary sodium hydrogen phosphate and citric acid was used for the buffering solution, the total ionic strength of which was adjusted to 0.5 by adding sodium chloride. The subsolutions of pH above 8 or below 2 were prepared by using sodium hydroxide or hydrochloric acid, respectively, instead of the buffering solution mentioned above. pH measurements were made using a pH meter with a glass electrode.

The plots of A_i and A_v against pH are given in Fig. 1, where it is seen that both

References p. 177.

A_i and A_v vary only slightly with pH in the pH range 2~7, and rise steeply at less than 2 until they reach a maximum value, 18 m^2/mg at pH 0.2. This result is not parallel with the extent of spreading against the pH, which is known from the surface pressure measurement, since a plot of the extent of spreading, as measured

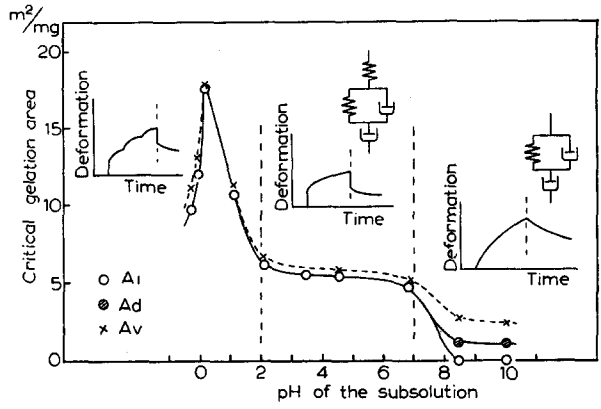

Fig. 1. The effect of pH of the subsolution upon the critical gelation area of ovalbumin monolayers. Insets indicate respectively the deformation *vs.* time curve in the pH region 10 to 7, 7 to 2, and below 2.

by GORTER's so-called limiting area, against the pH of the subsolution yields a W-shaped curve, the maximum area coinciding with the isoelectric point of the protein[6]. The mechanical model of gelled films was, as shown in the insets of Fig. 1 represented by a four-element model involving instantaneous elasticity, delayed elasticity, and simple viscosity in the pH range 0~8, whereas it was represented by a three-element model without instantaneous elasticity at pH values more than 8.4.

It is generally known that gel formation in bulk is brought about by the development of a three-dimensional network in the volume of the solution. Likewise, the gelation of films is probably due to the growth of a two-dimensional network structure throughout the whole surface film. Therefore the critical gelation area may be determined not only by the binding forces between film molecules, but also by the degree of extension or the shape of them. It is obvious that these factors are dependent upon the subsolution. It is considered that with the increase or decrease of pH from the isoelectric point, Coulomb electric repulsion between the ionized groups of ovalbumin molecules prevents the formation of intermolecular bonds, owing to the high net charge on the molecule, leading to the decrease of the critical gelation area. The possibility should also be considered that protein films on extremely acidic or alkaline subsolution may undergo hydrolytic decomposition. In fact, Fig. 1 indicates that gelation fails to occur on the strong acidic or alkaline subsolution. However, in order to interpret the maximum gelation area at acidic region, another factor should also be considered. It is probable that the pH of subsolution influences the intra- as well as the intermolecular bonds. Consequently, it is expected that the shape of film molecules varies with the pH of subsolution. Recently IMAHORI[7] presented evidence, from the surface diffusion experiments, that ovalbumin molecules spread on the acidic subsolution have an extremely elongated shape. He has shown that the axial ratio of surface-denatured ovalbumin molecules, assumed to have the shape of elliptical disks, is 30:1 at pH 1 and 15:1 at pH 3. It is possible that, with the compression of the film these elongated molecules are allowed to contact each other end-to-end or end-to-side, forming a loose network structure by intermolecular bonds, though

References p. 177.

the nature of bonding forces is obscure. This is also indicated from the fact that the surface gelation occurred at 5~18 m^2/mg, which is far larger than the co-area of film molecule of ovalbumin (*ca.* 1.0 m^2/mg).

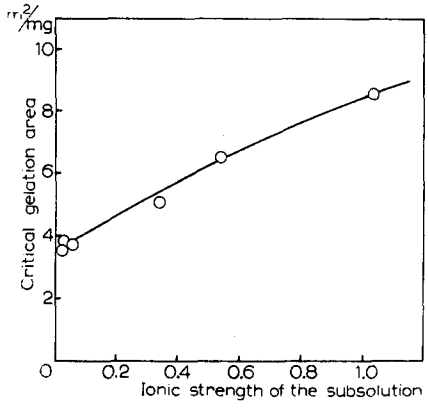

Fig. 2. The effect of the ionic strength of the subsolution upon the critical gelation area of ovalbumin monolayers.

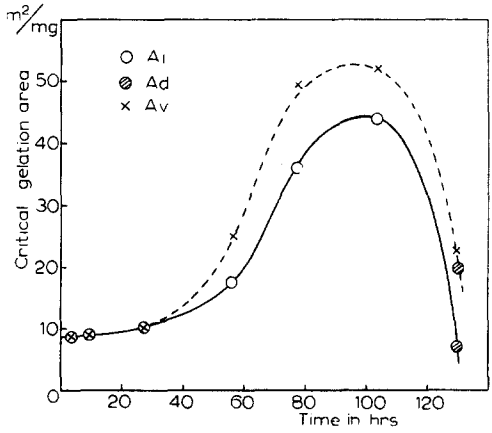

Fig. 3. The critical gelation areas of monolayers spread from ovalbumin solutions that have been stored for various periods.

Next, the concentration of sodium chloride in the buffer solution at pH 4.8 was varied in order to investigate the effect of ionic strength on the gelation. As shown in Fig. 2, it was found that the critical gelation area increases with the ionic strength of subsolution. This tendency is quite parallel with the extent of spreading[6].

2. *Spreading solution*

A. *Aging effect.* A 0.0102% solution of ovalbumin was stored at 0~2° C immediately after preparation in order to prevent bacterial contamination, and at various intervals films of ovalbumin were spread onto 5% ammonium sulfate solution for the critical gelation area to be measured. The result is shown in Fig. 3, where it is seen that both A_i and A_d increase with aging time until they reach a maximum value at 100 hours. It was observed that this effect proceeds more rapidly with the solution stored at a room temperature. On the other hand, such aging effect was not seen in the surface pressure–area relationships. Our results suggest that a certain amount of aging of the ovalbumin solution facilitates the unfolding of ovalbumin molecules at the air–water interfaces. It may be assumed that the observed effect is associated with a change in the ovalbumin solution that has occurred during storage. Recently, BIER AND NORD[8] secured evidence by means of the light-scattering method that these ovalbumin molecules undergo association during aging. Although it is not clear how far this association is connected with our observation, our experiment also indicates that the ovalbumin solution is a very labile system. Further study must be carried out to elucidate the nature of these phenomena.

B. *The addition of organic spreader.* Some investigators[9,10] have reported that an excellent spreading solution for protein is aqueous propyl alcohol with a small amount of sodium acetate or aqueous amyl alcohol. However, it is not definitely established that the film spread from such spreading solution is that of free protein.

References p. 177.

We prepared similar spreading solution by dissolving ovalbumin in aqueous solution of propyl alcohol (*n*- and *iso*-), amyl alcohol (*n*- and *iso*-), acetone and pyridine respectively, and spread the films from their solution on to a buffer solution of pH 4.5.

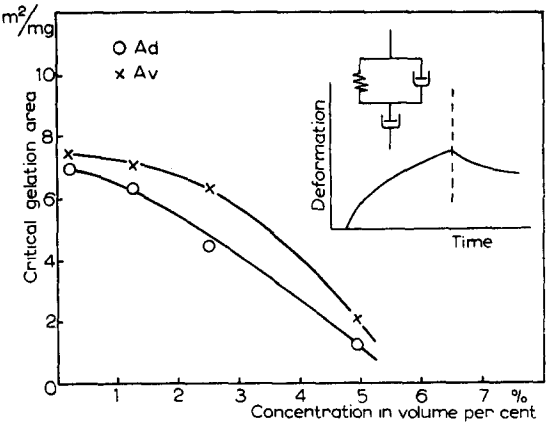

Fig. 4. The effect of *iso*amyl alcohol on the critical gelation area of ovalbumin monolayers. Subsolution; 0.15 *M* NaCl, 1/500 *M* phosphate buffer, pH 4.8, temp. 15° C.

A remarkable effect on the viscoelastic behavior of the films was observed, though identical surface pressure-area relationships were obtained independent of the existence of organic additive in spreading solutions. The ovalbumin films from solution free of organic spreaders, as mentioned already, exhibited distinct instantaneous elasticity, while those from solution containing organic spreaders failed to exhibit any instantaneous elasticity even when compressed down to gel state, showing delayed elasticity alone. The critical gelation area A_d or A_v was found to decrease with increasing concentration of organic spreader. A typical example with *iso*amyl alcohol is given in Fig. 4. It should be noted that the nature of the films from such a spreading solution is quite different from those from the spreading solution without organic spreader. The present result could be interpreted by the idea that some groups of ovalbumin molecule responsible for the formation of a network structure are bound by the added alcohol, which acts as a "plasticizer". This is quite consistent with GORTER's view[6] that the film obtained from aqueous alcohol solution is not to be considered as a film of the protein itself, but rather of alcohol-protein complexes.

SUMMARY

1. The critical gelation area of ovalbumin monolayers was determined over the pH range (o~11) of the subsolution and was shown to be a maximum at pH 0.2. This result was discussed in connection with the shape of film molecules.

2. The change undergone by ovalbumin solution during aging was followed by a surface rheology technique.

3. Organic additives as spreaders in the spreading solution had a considerable effect on the rheological characteristics of protein monolayers. This fact suggests the formation of organic additive-protein complexes.

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