The Roles of Prolyl Residue in Polypeptide Monolayers. III. Surface Films of Gelatin

By Shoichi IKEDA and Toshizo ISEMURA

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Collagen and gelatin are unusually rich in prolyl and hydroxyprolyl residues as compared with ordinary globular proteins. Because of the specific structures of these imino acid residues, the configurations of polypeptide chains in these proteins are expected to be quite different from those in other proteins. In fact, such characteristics have been observed in solutions of collagen[1-3] and gelatin[4,5] as well as in collagen fiber[6]. In surface films, if they are spread on aqueous surfaces, collagen and gelatin will likewise show a characteristic behavior.

It was established with synthetic polypetides[7,8] that the presence of prolyl residues in a polypeptide chain makes their surface films more expanded, chiefly due to the decrease in number of hydrogen bonds and, accordingly, the condensed nature of films of ordinary synthetic polypeptides may be ascribed to the hydrogen bonding in films. It has been noticed that most globular proteins form condensed films. If the roles of prolyl residue in polypeptide monolayers are reproduced in protein films, collagen and gelatin should form expanded films on aqueous surfaces.

In the present paper it will be examined by the measurements of surface pressure, potential and viscosity, whether these considerations are valid or not for the surface films of gelatin.

Experimental

Materials.—Gelatin was purified from leaf gelatin of Nihon Hikaku Co., Ltd., by desalting it in acetate buffer at pH 4.8 and then washing it with distilled water. The pH of the solution of gelatin thus obtained was 4.9. Concentrations of gelatin solutions were determined by measuring the dry weight of 0.5% stock solution. Spreading solutions were prepared by diluting the stock solution ten times with distilled water.

Methods. — Surface pressure, potential and viscosity were measured by a surface balance of float type, a vibrating electrode and an oscillating rotatory disk, respectively. Surface moment was calculated from surface potential, using the Helmholtz equation.

To adjust pH of aqueous subphases to 2.0, 5.6 and 11.2, 0.01 N hydrochloric acid, distilled water and 0.01 M potassium carbonate were used, respectively. Purified potassium chloride was added to prevent the film from dissolving into aqueous subphases.

Results

Fig. 1 shows surface pressure-area (Π-A) and surface moment-area (μ-A)

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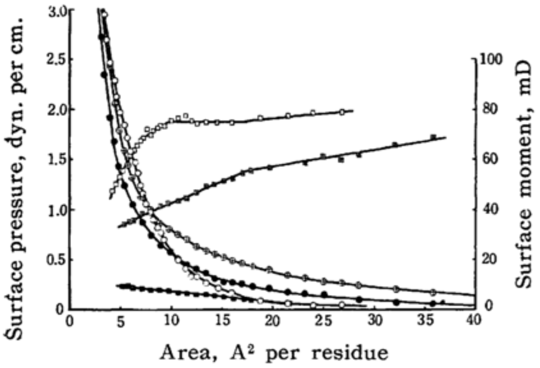

Fig. 1. Surface pressure and moment vs. area curves of gelatin on salt-free solutions at pH 2.0 (⊕, ⊞), 5.6 (○, □) and 11.2 (●, ■): 20°C.

curves of gelatin films on salt-free subphases at three different pH's. Surface pressures of gelatin films on distilled water were scarcely influenced by the temperature of spreading solution and the nature of spreading solvents. Besides a gelatin solution in distilled water at room temperature, a solution in distilled water at 48°C, and solutions in 0.1 M potassium rhodanate, in 40% ethanol and in a mixture of dichloroacetic acid-isopropanol-water (5 : 2 : 3) were examined. Fig. 2 shows the Π-A and μ-A curves of gelatin films on 1 M potassium chloride solutions at three different pH's. On the alkaline side the film had a slight tendency to dissolve into aqueous subphase, when spreading solution was freshly prepared. The surface viscosity-area (η-A) curve of the film on 1 M potassium chloride is also shown in Fig. 2.

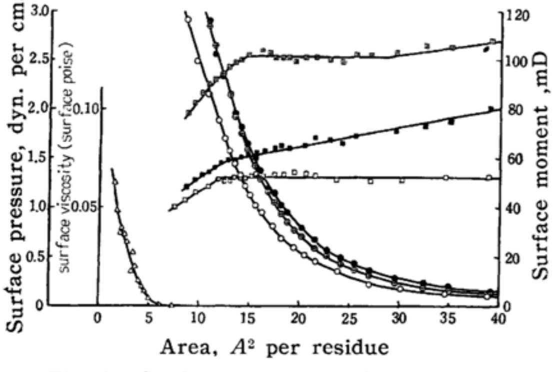

Fig. 2. Surface pressure and moment vs. area curves of gelation on 1 M potassium chloride at pH 2.0 (⊕, ⊞), 5.6 (○, □) and 11.2 (●, ■), and surface viscosity vs. area curve on 1 M potassium chloride at pH 5.6 (△): 20°C.

Discussion

Both collagen and gelatin are composed of about 30% glycyl residues and about 30% prolyl and hydroxyprolyl residues. In solution a collagen molecule exists as a rigid rod-like particle[1,2] which is also found in collagen fiber from X-ray diffraction studies[6]. On the other hand, a gelatin molecule behaves as a random coil like ordinary polymer molecules but unlike a collagen molecule[4,5].

It was reported, however, that, if collagen and gelatin are spread as surface films, they are indistinguishable from each other: collagen spread from its formic acid solution and gelatin from its aqueous and formic acid solutions give identical Π-A and μ-A curves on 30% ammonium sulfate solution[9]. This fact is consistent with the present observations that Π-A curves of gelatin films are independent of the nature of the spreading solutions.

In spite of its hydrophilic nature, gelatin formed a stable film on distilled water, as already noted[10]. The extent of the spreading of film was very incomplete and not very much influenced by the acididity and alkalinity of aqueous subphases. Such behavior of gelatin film is quite different from those of other protein films[11]. Further, molecular expansion of gelatin as was observed in aqueous solutions[12] was not reflected in its surface films. The surface moment of gelatin was higher on distilled water at pH 5.6 than on aqueous subphases at both sides of this pH. This is not consistent with the idea of titration of surface films[13,11], but is in agreement with the result for collagen film on aqueous solutions, the pH's of which were adjusted by hydrochloric acid[9]. Addition of salt into aqueous subphases caused gelatin films to spread more[14]. Even on 1 M potassium chloride, however, gelatin spread still incompletely and appeared to dissolve on compression. The surface pressures at different pH's were nearly identical on such a concentrated salt solution, as was generally expected from the theory of ionized

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monolayers. Apart from these facts, all the surface films of gelatin were found to be of expanded types.

It has been observed that globular proteins, when spread as monolayers, form condensed films, at least, at the isoelectric point and occupy an area of about 15 A^2 per residue, which would mean that the protein films are in the β-configuration[15,11]. The expanded nature of gelatin films may be attributed to the roles of prolyl residues such as are found in the polypeptide monolayers, i. e. to the reduced number of hydrogen bonds[7]. In surface viscosity behavior was exhibited the general property of expanded films showing that the surface viscosity is only manifest at high surface pressure region[8]. The incomplete spreading and pressure-solubility of gelatin films may come from its hydrophility and specific configuration of polypeptide chains.

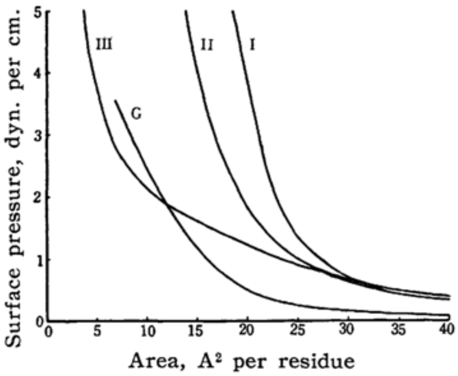

Fig. 3. Comparison of surface pressure-area curve of gelatin with those of some synthetic polypeptides. G: gelatin, I: poly-L-prolyl-L-leucylglycine, II: copoly-1:1:1-(L-proline, L-leucine, DL-alanine), III: poly-L-proline. G and III on 1 M potassium chloride.

Collagen and gelatin molecules were once regarded as consisting of L-prolyl-glycyl-R sequences, on which basis Astbury and Bell[16] suggested a chain configuration in collagen fiber. Recently, Millionova and Andreeva[17] proposed from their X-ray study on poly-glycyl-L-proline that glycyl-L-prolyl sequences are repeated in a collagen molecule. The amino acid analysis on partial hydrolysates of gelatin[18] and collagen[19] has, however, shown that a considerable amount of L-prolyl-L-hydroxyprolyl sequences are found in them, and now it is generally recognized that gelatin and collagen contain these sequences abundantly. At present, a configuration of polypeptide chain in collagen fiber has been established on the basis of the repeated structure of L-prolyl-L-hydroxyprolyl-glycyl sequences[6].

It appears that the surface pressure behavior of gelatin film also supports the presence of these sequences. In Fig. 3 the Π-A curves of some polypeptide films reported in a previous paper[7] are summarized together with that of gelatin film on 1 M potassium chloride. It will be seen that the extent of expansion or the surface compressibility of gelatin film lies near that of copoly-1 : 1 : 1-(L-proline, L-leucine, DL-alanine) and is lower than that of poly-L-proline. In the former copolypeptide L-prolyl-R-L-prolyl sequences are possible and in the latter polypeptide the successive L-prolyl residues exist. Thus, it is considered that a gelatin molecule consists of L-prolyl-R-L-prolyl or L-prolyl-L-prolyl-R sequences. Since some of the prolyl residues in gelatin are substituted by hydroxyprolyl residues and the presence of hydroxyprolyl residues makes a surface film somewhat condensed, the latter sequence, i. e. L-prolyl-L-hydroxyprolyl-glycyl, may be favorable to explain the extent of expansion of gelatin film, in accordance with the results of chemical analysis.

If L-prolyl-L-hydroxyprolyl bonds are assumed to exsist in a gelatin molecule, the effect of salt on gelatin films can be well interpreted following Harrington's suggestion[20], that most of these bonds are *trans* in distilled water and the addition of salt causes the *trans-cis* transformation of these bonds. If the parts of *cis* bond in a gelatin molecule remain on the surface while the parts of *trans* bond dissolve in aqueous phase and the other parts are either on or in aqueous subphase according as their own hydrophility and their surrounding chain configurations, it can be explained why gelatin can form a stale film even on distilled water in spite of its hydrophilic nature. Further, the presence of salt in subphases would stabilize the *cis* bond more than the *trans* bond, causing the *trans-cis* transformation, and make

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films spread more easily. There considerations are consistent with those made for poly-L-proline film previously7).

Summary

Surface films of gelatin, which is very rich in L-prolyl and L-hydroxyprolyl residues, were examined on salt-free and 1 M potassium chloride solutions at three different pH's by the measurements of surface pressure, potential and viscosity. They were found to spread incompletely but to be of expanded types. The incomplete spreading and the other properties of the films were regarded as a manifestation of the specific configuration of gelatin in films. The expanded nature of the films was attributed to the roles of prolyl residue in gelatin such as observed in polypeptides. The extent of expansion of the films appeared to give evidence to support the results from chemical analysis that a gelatin molecule is composed of a large amount of L-prolyl-L-hydroxyprolyl bonds. The behavior of gelatin films was adequately explained by assuming that the parts of *cis* L-prolyl-L-hydroxyprolyl bond form the film and the parts of *trans* bond dissolve in aqueous subphase.

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Faculty of Science
and
Institute for Protein Research
Osaka University
Osaka