The Interaction of Proteins with Silicic Acid

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Although silicon is a constituent of normal animal tissues, silicic acid formed by the dissolution of inhaled quartz particles can induce the formation of pathological lesions in which the normal tissue is replaced by fibrotic tissue arranged concentrically around the silica particles. The mechanism of the change which constitutes the disease, silicosis, is unknown. Schulman & Rideal (1937) demonstrated the interaction of a protein with silicic acid by injecting the acid beneath a monolayer of gliadin at pH 6.2. The silicic acid was adsorbed to the protein layer and after 12 hr. produced a film which could not be penetrated or dispersed by sodium oleate.

This communication describes a more extensive study designed to investigate the conditions under which proteins react with silicic acid and to determine which of their functional groups are responsible for the interaction. Two methods of study were used. Protein monolayers were spread on silicic acid solutions and the characteristics of the films were compared with monolayers on subsolutions which contained no silicic acid. Silicic acid and protein solutions were also mixed in bulk and their interaction was followed turbidimetrically. The two proteins examined, bovine serum albumin and methaemoglobin, were chosen because of the considerable difference between their isoelectric points.

The properties of gliadin films on silicic acid substrates indicate that the interaction involves two processes: (a) the adsorption of silicic acid to the protein, and (b) the polymerization of the silicic acid. When silicic acid, in the form of the monomer or low polymers, is adsorbed beneath a protein film, polymerization between molecules adsorbed on adjacent protein sites follows and results in the formation of a complete network underneath the protein film, which is thereby solidified. If, however, the protein is spread on a solution containing silicic acid already in the form of high polymers and adsorption occurs, the protein is anchored by the silicic acid and is prevented from spreading completely to a monolayer. If interaction takes place, the area to which the protein will spread may be considerably decreased.

The rate of polymerization of silicic acid depends markedly on pH and, except in the region pH 5.5–6, it is slow. To simplify the interpretation of our measurements and to allow a study of protein-silicic acid interaction without the complicating

polymerization reaction, the silicic acid sols were pre-polymerized at pH 6 before use as subsolutions.

A parallel study of the interaction of monolayers of a carboxylic acid, an alcohol and an amine allows some deductions to be made as to which groups of the protein are likely to interact with silicic acid. The influence of pH on the formation of silicic acid-protein complexes is shown most clearly by turbidimetric measurements on bulk solutions.

MATERIALS

Silicic acid. A sample of pure vitreous silica, kindly supplied by the Thermal Syndicate Ltd., was crushed, mixed with 4 times its weight of Na_2CO_3 and fused in a platinum crucible. The melt was dissolved in distilled water. The concentration of the solution was adjusted to about 0.15 M and the solution was brought to pH 6 (glass electrode) by titration with 2N-HCl. Unless stated otherwise, all silicic acid solutions were first polymerized at pH 6 and at a concentration of 0.1 M for 40 min. before bringing to the required concentration and pH. (Concentrations have been expressed throughout as molarities with respect to $\text{Si}(\text{OH})_4$.) Sols of silicic acid are polydisperse and their composition cannot be determined; for a discussion on the state of these sols see the Discussions of the Faraday Society on Colloidal Electrolytes (1935).

Methaemoglobin. This was prepared from human erythrocytes which were washed five times with isotonic saline then lysed with distilled water. The haemoglobin content was estimated and a 20% molar excess of 0.7M- $\text{K}_3\text{Fe}(\text{CN})_6$ added.

Poly-L-lysine. The sample was kindly supplied by Dr C. H. Bamford of Courtaulds Ltd., Maidenhead. It was prepared by the Leuchs reaction, as described by Frankel, Grosfeld & Katchalski (1948), and it had a degree of polymerization of 30.

Octadecan-1-ol (m.p. 57°). This was supplied by British Drug Houses Ltd. Its purity was checked by following its force/area curve when spread on 0.01M-NaCl. The curve corresponded to that obtained by Adam & Dyer (1924).

Bovine serum albumin. A crystalline preparation was supplied by Armour Biochemicals Ltd.

Heptadecylamine hydrochloride was kindly prepared by Dr C. L. Leese from stearic acid by the Schmidt reaction (Briggs, De Ath & Ellis, 1942). The sulphate was converted into the free base by the addition of NaOH. The base was extracted with petroleum ether. Heptadecylamine hydrochloride, m.p. 168°, was precipitated from the solution by dry HCl.

Spreading solvents were kept over activated charcoal for several hours before being filtered and redistilled to remove surface-active impurities.

Water, after distillation, was passed through a mixed-bed resin (Permutit Biode-minerolit) ion-exchange column in a Polythene tube. Its conductivity was less than $2 \times 10^{-7} \Omega^{-1} \text{ cm}^{-1}$. All other reagents were of A.R. grade.

EXPERIMENTAL AND RESULTS

Monolayers were studied on a standard Langmuir-Adam trough. The trough itself was cut from block Polythene, and was waxed. The whole apparatus was encased to exclude dust and to provide thermal insulation. Thermostatic control kept the temperature of the subsolution constant within $\pm 0.5^\circ$. The sensitivity of the apparatus was 0.1 dyne/cm .

Turbidity measurements were made in a simple turbidimeter (Holt & Chalk, 1953) consisting of a source of white light mounted to send a beam through one of two glass cells, either of which could be slid into the light path. That part of the light which is scattered at about 90° to the incident beam falls on a photoelectric cell set above the glass cells; the output of the photoelectric cell is measured by a sensitive galvanometer.

Heptadecylamine. Heptadecylamine was first spread as the free base, but, because its solutions rapidly combined with CO_2 , the hydrochloride was preferred. Films spread from the free base gave force/area curves identical with those given by the hydrochloride. The amine or its hydrochloride was spread from 0.07% (w/v) solution in benzene-methanol (5:2, v/v) on to subsolutions (0.01 M-NaCl , 0.005 M-NaHCO_3 ; pH adjusted with HCl or NaOH). After the films had been studied over a pH range from 3 to 12 on these substrates, the amine was spread on solutions containing, in addition to the 0.01 M-NaCl and 0.005 M-NaHCO_3 , 0.005 M silicic acid. To compare the effects of different buffers, a 0.005 M sodium phthalate buffer was also used at pH 6.2. After the heptadecylamine had been applied to the surface, films were retained at an area of $74 \text{ sq.}\text{\AA}/\text{molecule}$ for 5 min. before measuring the force/area characteristics.

The force/area curves of heptadecylamine on silicic acid-free subsolutions are shown in Fig. 1. On alkaline solutions the films were condensed to areas, under zero compression, of between 27 and $29 \text{ sq.}\text{\AA}/\text{molecule}$, while from pH 6 to 8 they were even more closely packed, giving a value of $24.5 \text{ sq.}\text{\AA}/\text{molecule}$ at pH 7.1. Below pH 6 they were vapour-expanded. At all pH values the films were stable up to approximately 37 dynes/cm., at which pressure they were compressed to about $20 \text{ sq.}\text{\AA}/\text{molecule}$. With phthalate buffer at pH 6.2 the film could not be compressed below $35 \text{ sq.}\text{\AA}/\text{molecule}$ before collapsing at 35 dynes/cm.

The force/area curves of the amine on silicic acid subsolutions are shown in Fig. 2. Above pH 10 they appear to be identical with those of the amine on silicic acid-free subsolutions. At pH 9 the films were expanded and a surface pressure of just over 50 dynes/cm. was required to compress them to $20 \text{ sq.}\text{\AA}/\text{molecule}$. From pH 9 to 5 the films were partly solidified at large areas/molecule and on compression they collapsed at about 15–20 dynes/cm. leaving shearing films. Below pH 5 no inter-

Fig. 1. Force/area curves of heptadecylamine (0.01 M-NaCl , 0.005 M-NaHCO_3), 20° . pH: $\blacksquare$, 9.0; $+$, 7.2; $\circ$, 5.5; $\times$, 5.1; $\blacklozenge$, 4.4; $\bullet$, 4.0; $\blacktriangle$, 3.0; $\blacktriangledown$, 6.2 on 0.005 M phthalate buffer.

Fig. 2. Force/area curves of heptadecylamine (0.01 M-NaCl , 0.005 M-NaHCO_3 , 0.005 M silicic acid), 20° . pH: $\circ$, 11.0; $\blacktriangledown$, 9.9; $\square$, 9.0; $\triangle$, 8.1; ∇ , 6.6; $\times$, 5.7; $\bullet$, 5.0; $+$, 4.2; $\diamond$, 3.4.

Fig. 3. The areas of heptadecylamine monolayers measured at 5 dynes/cm. plotted against the pH of the subsolution: I, subsolution (0.01 M-NaCl , 0.005 M-NaHCO_3); II, subsolution (0.01 M-NaCl , 0.005 M-NaHCO_3 , 0.005 M silicic acid).

action was apparent and the vapour-expanded films deviated very little from those on silicic acid-free solutions.

Although the subsolution contained excess of silicic acid, there was a variation in the area/molecule in the solidified film when the quantity of amine spread was varied. It follows that the amount of silicic acid adsorbed depends on the initial state of expansion of the film, as would be expected if polymers are adsorbed from a polydisperse system. (Compounds have been prepared by other methods (Merrill & Spencer, 1951) in which guanidine and quaternary bases are combined with silicic acid in stoichiometric proportions.)

For this reason all films were held at a fixed area of 74 sq.Å/molecule for 5 min. before compression. Therefore, the measured values of the area/molecule in the compressed film relate only to the particular experimental conditions used.

Fig. 3 shows the variation of molecular area of the amine with pH, measured at a surface pressure of 5 dyne/cm. and compares the areas on silicic acid with those on silicic acid-free solutions.

Myristic and stearic acids. The acids were spread from solutions in light petroleum (b.p. 60–80°) on to subsolutions of 0.05M-NaCl or (0.05M-NaCl, 0.025M prepolymerized silicic acid). Myristic acid was used for pH values up to 4 (above this value the films are too soluble to form stable monolayers) and stearic acid at pH 6.7.

Fig. 4. Force/area curves of myristic acid on 0.05M-NaCl.

Fig. 5. Force/area curves of myristic acid (0.05M-NaCl, 0.025M silicic acid), 20°.

The force/area curves for myristic acid were unaltered by silicic acid (Figs. 4, 5), and there were no signs of solidification even after 12 hr. On silicic acid-free subsolutions at pH 6.7, stearic acid forms condensed films, the force/area curves of which are divisible into two linear regions, above and below 20 dynes/cm. Although silicic acid partially eliminated the lower slope so that complete compression occurred at low pressures, there was no tendency for the film to become solidified (Fig. 6).

Octadecan-1-ol. The spreading solvent used was light petroleum. Subsolutions were 0.2M-NaCl and (0.2M-NaCl, 0.1M silicic acid), both at pH 6. In this case the silicic acid was not prepolymerized. The films were compressed to 45 sq.Å/molecule for 40 min. before the force/area characteristics were determined.

Curve I of Fig. 7 shows the state of the alcohol film on the sodium chloride alone (after 40 min.), and curve II on the sodium chloride-silicic acid substrate after the same time. After 40 min. it was found that the film on silicic acid was in a solid gel stage, stable to more than 50 dynes/cm. surface pressure and reversibly compressible, but unable to expand to an area greater than the original 45 sq.Å/molecule. This result, although repeated, was under suspicion since it was possible that by using such a high silicic acid concentration, gelling may have occurred at the surface of the subsolution and the effect on the alcohol film could have been simply mechanical. Therefore the experiment was repeated, making certain that the time between preparing the silicic acid and spreading the alcohol

Fig. 6. Force/area curves of stearic acid at pH 6.7, 25°. I, subsolution (0.02M-NaCl); II, subsolution (0.02M-NaCl, 0.01M silicic acid).

film was as short as possible, i.e. less than about 15 min., so that gelling was unlikely to occur during the experiment. Treated in this way, the films were not solid even after 1 hr., but on the other hand they were stable up to 50 dynes/cm., whereas 40 dynes/cm. is the normal collapse pressure.

Methaemoglobin. Methaemoglobin, dissolved in 0.01M-NaCl in 1% (v/v) aqueous ethanol was

Fig. 7. Force/area curves of octadecan-1-ol, 20°. I, sub-solution (0.2M-NaCl); II, subsolution (0.2M-NaCl, 0.1M silicic acid).

Fig. 8. The areas of methaemoglobin monolayers measured at 5.5 dynes/cm. plotted against the pH of the subsolution, 20°. I, subsolution (0.05M-NaCl, 0.005M-NaHCO₃); II, subsolution (0.05M-NaCl, 0.005M-NaHCO₃, 0.025M silicic acid).

spread on to (0.05M sodium chloride, 0.005M sodium bicarbonate). Spreading was complete in 15 min. Surface pressure/area curves were determined at a number of pH values. The variation of area with pH at the constant surface pressure of 5.5 dynes/cm. is shown in curve I (Fig. 8). Two minima occur, one at pH 4.3 and the other at pH 11.0. Between these points is a region in which the film characteristics show little variation.

Methaemoglobin was spread on similar subsolutions containing also 0.025M silicic acid and the force/area characteristics were again measured after 15 min. Between about pH 4.5 and 9 spreading was incomplete and the apparent area/mg. was lower than for methaemoglobin on silicic acid-free subsolutions. The films, however, were stable at surface pressures above the normal value of about 22 dynes/cm. These apparent areas/mg. (computed from the mass of protein spread) are compared with the normal in Fig. 8.

Turbidimetric study of the influence of pH on the interaction of silicic acid and proteins

The protein or polypeptide (10 ml. of 0.1% (w/v) aqueous solution) was mixed with an equal volume of 0.025M pre-polymerized silicic acid. The pH was adjusted by the addition of NaOH or HCl and the volume made up to 25 ml. The light-scattering power of each suspension was then compared in the turbidimeter with that of the original silicic acid sol in comparable dilution.

As methaemoglobin is itself precipitated in acid solution, turbidity measurements were also made on a series of methaemoglobin solutions.

The turbidimeter readings of the several protein-silicic acid complexes are plotted against pH in Fig. 9, the readings being expressed as a fraction of the maximum value obtained for each protein.

Fig. 9. The turbidities at various pH values of solutions containing protein or polypeptide to which silicic acid had been added. For details, see text.

I Poly-L-lysine	} With silicic acid
II Methaemoglobin	
III Serum albumin	
IV Methaemoglobin alone	

Bovine serum albumin. The turbidity was greatest at pH 4.6 and was immediately dispersed by an increase or decrease of 2 units of pH.

Methaemoglobin. The maximum turbidity occurred at pH 6.3–6.8. Another peak appearing at pH 3.1 coincided with that of methaemoglobin in the absence of silicic acid and is not to be regarded as due to co-precipitation of haemoglobin with silicic acid.

Poly-L-lysine. No precipitate was formed below pH 6. Unlike the rounded maxima given by the proteins, polylysine gave a sharp peak at pH 9.

DISCUSSION

Of the simple compounds studied as surface films, only heptadecylamine shows a definite interaction with polysilicic acid. The dependence of area/molecule of amine on the size of the buffering anion, demonstrated when the phthalate buffer was substituted for a carbonate buffer, indicates that the amine is associated with the anion at pH 6, and from curve I, of Fig. 3, it is evident that the amine salt is associated in the pH range 5–9. It is over this range that silicic acid is adsorbed, suggesting that the adsorption complex is an insoluble amine silicate. The concentration of silicate ions at this pH would be low (pK_a of silicic acid = 9.5, Treadwell, 1935), but their removal from the system to form the insoluble silicate layer would shift the equilibrium and induce further ionization of the acid. The formation of an insoluble silicate must then represent the main type of interaction between proteins and silicic acid. No interaction is apparent between silicic acid and the carboxyl groups.

From the turbidimetric measurements it can be seen that the interaction of silicic acid with methaemoglobin is a maximum at pH 6.5–7 and with serum albumin at pH 4.6. The surface-area measurements on the haemoglobin films indicate that a maximum interaction occurs at pH approx. 6. These values are close to the isoelectric points of the two proteins (haemoglobin, 6.8; serum albumin, 4.9), and they suggest that silicic acid is bound most strongly when the net charge on the protein is zero. On the alkaline side of its isoelectric point, the overall negative charge borne by the protein will act as a potential barrier to the approaching anions and will therefore effectively prevent any interaction. This is demonstrated by the effect of polysilicic acid on polylysine. The synthetic polypeptide bears no acidic groups other than a few α -carboxyl radicles, and it is evident that the ϵ -amino groups are able to react at pH values near 9, whereas in the protein the ionized carboxyl groups prevent interaction at this pH. Thus in the protein most of the lysine and arginine cannot react with silicic acid because of the repellent effect of the ionized carboxylic, phenolic and thioalcoholic groups. On the acid side of the isoelectric point the ion pairs are dissociated.

Judging by the pK_a values of the various amino acids represented in the side chains of the proteins, it seems probable that silicic acid is bound to the protein mainly by the histidine and α -amino groups.

The results of the measurements on alcohol films are not conclusive. They do not establish that silicic acid is adsorbed at an appreciable rate but they do suggest some interaction, in that the silicic acid appears to gel initially along the surface of oriented hydroxyl groups. Therefore, although silicic acid must be held mainly by the amino groups of the protein, secondary forces such as weak interactions with hydroxyl groups may also be possible. The cationic groups of the protein will attract silicate ions to the surface. The hydroxyl group cannot attract the silicic acid or silicate ions; they must reach the protein layer by diffusion and, consequently, any interaction will be a much slower process. It would seem then that the reactants are brought together primarily by the forces of electrovalency but are later anchored by subsidiary links, such as hydrogen bonds and the van der Waals forces. The analogy between silicic acid and the polysaccharidic acids is evident.

SUMMARY

1. The behaviour of methaemoglobin, heptadecylamine, myristic acid, stearic acid and octadecan-1-ol monolayers spread on buffers with and without silicic acid have been compared. The interactions with silicic acid of bovine serum albumin, methaemoglobin and poly-L-lysine were studied turbidimetrically.

2. The proteins and polylysine bind silicic acid most strongly at their respective isoelectric points.

3. Heptadecylamine reacts with silicic acid between pH's 5 and 9. Octadecanol perhaps reacts weakly. The carboxylic acids do not react.

4. It is concluded that proteins combine with silicic acid mainly through their amino groups but subsidiary links, such as hydrogen bonds and the van der Waals forces, may also be involved.

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