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# Bonding in Protein and Polypeptide Monolayers

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## SUMMARY

The logarithm of the surface viscosity of protein and polyamino acid monolayers was found to be a linear function of the surface pressure in agreement with the Moore-Eyring theory. The area of the flow unit was similar for all proteins and corresponded to a segment of the molecule of about seven or eight amino acid residues. The free energies of activation for flow at pH 5.5 were fairly constant, falling between 15.6 and 16.6 kcal mole$^{-1}$ of flow unit. Evidence was obtained for ascribing a large part of the free energy of activation to the breaking of intermolecular keto-imido hydrogen bonds. The interactions of protein monolayers with mercuric ion in the subphase and with lipid incorporated in the monolayer are discussed.

An air/water interface acts as a "good solvent" for proteins, adsorption leading to unfolding of protein molecules. Many of the bonds which stabilize the molecule in its aqueous solution state are thereby broken. These bonds may re-form intermolecularly and this is favored by the relatively high concentrations possible in the monolayer. Intermolecular bonds are detected most readily by surface viscosity measurements and the importance of the different types of bond may be assessed by a comparison with selected polyamino acids.

The best known theoretical treatment of surface viscosity is that of

Moore and Eyring [1]. This is an extension to two dimensions of the treatment for ordinary viscosity by the Absolute Reaction Rate Theory. The flow of the monolayer is treated as a movement of flow units, normally molecules, from one equilibrium position to another, passing over an intermediate activation energy barrier. The most simple expression for the surface viscosity ($\eta_S$) is then:

$$\eta_S = \frac{h}{A} \exp \frac{\Delta G}{kT} \tag{1}$$

where h = Planck's constant, A = area per flow unit, and $\Delta G$ = free energy of activation for flow. $\Delta G$ is associated with (1) the work required to form a hole in the surface sufficiently large for the molecule to move into, and (2) the work required to move the molecule into the hole. The second term includes the work required to break all the bonds which form with neighboring molecules.

If the monolayer is at a surface pressure, $\pi$, additional work has to be done against the surface pressure in order to create a hole. Therefore

$$\eta s = \frac{h}{A} \exp \frac{\Delta G}{kT} \exp \frac{\pi A}{kT} \tag{2}$$

or

$$\ln \eta s = \ln \left(\frac{h}{A}\right) + \frac{\Delta G}{kT} + \frac{\eta A}{kT} \tag{3}$$

This equation therefore predicts a linear relationship between the logarithm of the surface viscosity and the surface pressure. The area per flow unit may be calculated from the slope and the free energy of activation from the intercept.

## EXPERIMENTAL

The apparatus consisted of a surface balance in which surface pressure and surface viscosity could be measured simultaneously. Surface pressure was measured by a floating mica strip. Terylene threads and high purity paraffin wax were used to connect the strip to the sides of the trough. The surface viscosity was measured with a torsional pendulum viscometer having a waxed Lucite cylinder oscillating freely in the surface. The cylinder was in turn attached to a brass bob which was suspended from the spindle of a Magslip motor by a Nichrome wire of 62 cm length. Oscillations were induced by another Magslip motor connected electrically to the one on the

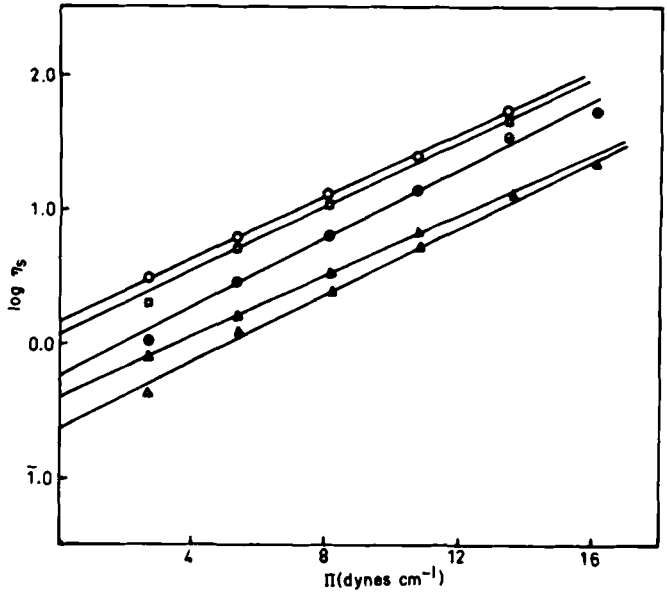


**Fig. 1.** Log $\eta_S$ vs $\pi$ for monolayers at pH 5.5. ○: Poly-DL-alanine. □: Human $\gamma$-globulin. ●: Pepsin. ▲: Bovine serum albumin. △: Lysozyme.

viscometer. Several different wires and brass bobs were used during the course of the experiments. The moment of inertia of the system was varied from 36 to 330 g cm$^2$ and the diameter of the suspension wire from 0.003 to 0.005 in.

Surface viscosity was calculated from Eq. (4):

$$\eta_S = \frac{2.303I\Delta\lambda}{2\pi P}\left(\frac{1}{a^2} - \frac{1}{b^2}\right) \tag{4}$$

where I = moment of inertia of oscillating system, P = period, $\Delta\lambda$ = difference in decadic logarithmic decrement between the monolayer covered and clean surfaces, a = radius of oscillating cylinder (0.5 cm), and b = radius of outer ring (9.0 cm).

Measurements were made only for relatively small damping of the oscillations. Under these conditions, the decrement and the period did not vary significantly with the amplitude of the oscillations.

Hydrochloric acid was used to obtain the low subphase pH while sodium hydroxide-boric acid buffers were used for the high pH region.

All water was distilled twice, shaken with activated charcoal, and filtered

before use. Monolayers were spread from aqueous solution by the method of Trurnit [2]. In some cases such as the protein-lipid mixed films, a small quantity of n-propanol was used in the spreading solvent.

The water in the trough was kept at 20 ± 0.5°C by circulating water from an external bath through a glass coil.

## RESULTS AND DISCUSSION

The logarithm of the surface viscosity was plotted against the surface pressure for a number of proteins and poly-DL-alanine (Fig. 1). Measurements were also made on poly-L-proline but, with the apparatus used, no surface viscosity was detectable even up to the highest surface pressure measured (20 dynes $cm^{-1}$).

Two features may be noted in Fig. 1; first, the plots follow closely a linear relationship in agreement with the Moore-Eyring theory and second, the slopes are similar for all the proteins. Values of A, the area per flow unit, and $\Delta G$ calculated from the slope and intercept respectively are shown in Table 1.

**Table 1.** Calculated Values of A and $\Delta G$ for Proteins

| Protein | MW ($\times 10^{-3}$) | A ($Å^2$) | $\Delta G$ (kcal $mole^{-1}$) |
|---|---|---|---|
| Polyalanine | 1.5 | 105 | 16.6 |
| γ-Globulin | 160 | 110 | 16.4 |
| Pepsin | 34 | 120 | 16.1 |
| Serum albumin | 70 | 100 | 15.8 |
| Lysozyme | 15 | 115 | 15.6 |

All values of A fall between 100 and 120 $Å^2$ which corresponds to a flow unit of seven or eight amino acid residues (cf. Ref. 3). It has been well-established from bulk viscosity studies that the unit of flow of many linear polymers is a segment of the molecule of approximately constant size. In agreement with these considerations of the flow unit, the activation energy for flow is also fairly constant, varying from 15.6 to 16.6 kcal $mole^{-1}$ for the proteins studied.

The result that the surface viscosity of poly-L-proline is undetectable is an important pointer for the interpretation of the surface viscosity of polypeptide monolayers. Previous workers [4] have shown that the

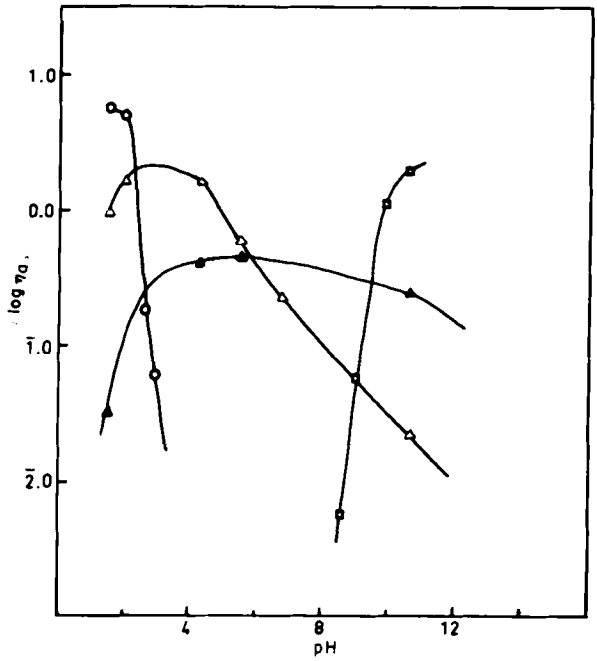


**Fig. 2.** Log $\eta_0$ vs pH for monolayers. ▲: Bovine serum albumin. △: Pepsin. ○: Poly-L-glutamic acid. □: Poly-L-lysine.

incorporation of prolyl residues into copolymers of amino acids drastically reduces the surface viscosity. Polyproline is unique among the polyamino acids in that there is no hydrogen atom on the peptide nitrogen so that keto-imido hydrogen bonds cannot form. It thus appears that the high surface viscosities of polypeptide monolayers and therefore at least a large part of the free energy of activation for flow arises from the breaking of these hydrogen bonds. If we assume a flow unit of seven amino acid residues, the maximum number of hydrogen bonds which could contribute to $\Delta G$ would be 7, remembering that each residue may form two bonds but that each bond is shared by two units.

## VARIATION OF SURFACE VISCOSITY WITH pH

The surface viscosities of pepsin and bovine serum albumin monolayers were measured as a function of pH. The results are shown in Fig. 2 together

**Table 2.** Effect of 0.001 M Mercuric Chloride Solution on $\eta_0$ ($\eta_S$ at $\pi = 0$) for Protein and Polypeptide Monolayers

| Polymer | Surface poise | |
|---|---|---|
| | Water | 0.001 M $HgCl_2$ |
| Serum albumin | 0.41 | 23.4 |
| Polyalanine | 1.5 | 1.5 |
| Polylysine | Not detectable | 2.4 |
| Polyglutamic acid | Not detectable | 1.8 |

with similar data for poly-L-glutamic acid and poly-L-lysine. The surface viscosity of protein monolayers is generally a maximum near the isoelectric point [5] as is seen in Fig. 2, although some proteins, notably γ-globulin [6] show little variation with pH. Near the isoelectric point, the number of salt-links would be expected to be a maximum, thus providing a possible explanation. However, from the behavior of the two ionizable polyamino acids, which cannot form salt-links alone, it seems more likely that the lowering of surface viscosity with increasing surface charge is caused by the breaking of hydrogen bonds as a result of the electrical repulsive forces set up in the surface.

It should be noted that although the surface viscosity of polylysine falls by more than a hundredfold on changing the pH from 9.9 to 8.7, the calculated free energy of activation only drops from 16.8 to 13.3 kcal $mole^{-1}$. The lower limit of the present measurements was about $10^{-3}$ surface poise. There is therefore a need for extending measurements well below this value in order to evaluate both the hydrogen bond breaking contribution and other contributions to the free energy of activation. Some refinement to the simple theory outlined above may also be needed to allow for non-Newtonian behavior. The use of monolayers has certain advantages over bulk materials in these studies. An oriented layer one molecule thick permits a simpler model to describe the flow properties.

## INTERACTIONS IN MONOLAYERS

Surface viscosity measurements are useful for detecting and interpreting interactions in monolayers between proteins and substances either dissolved

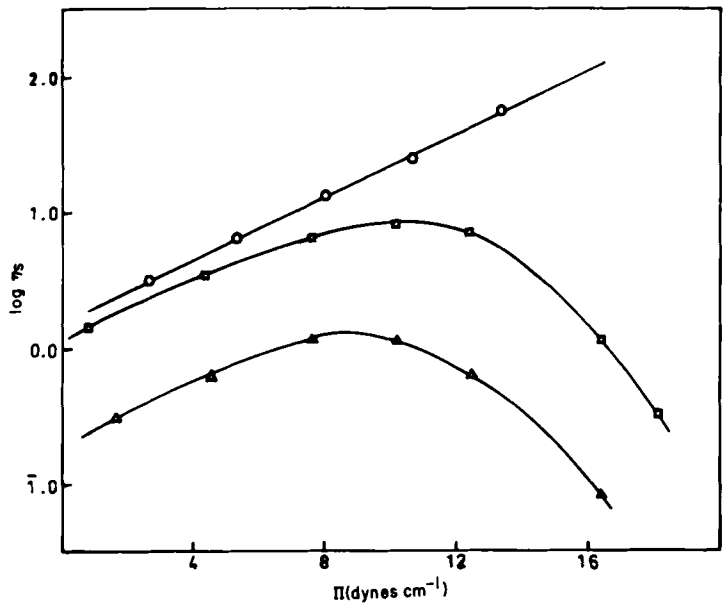


**Fig. 3.** Log $\eta_S$ vs $\pi$ for mixed monolayers of poly-DL-alanine and lecithin at pH 5.5. ○: Polyalanine. □: Polyalanine-lecithin 4:1 by weight. △: Polyalanine-lecithin 2:1 by weight.

in the subphase or incorporated in the monolayer. Table 2 shows some data for the surface viscosity of monolayers on a subphase of 0.001 M mercuric chloride solution. The increase in the surface viscosity of serum albumin by a factor of about 60 in the presence of mercuric ion is typical of a number of proteins which have been studied, others being insulin, ovalbumin, and hemoglobin. Mercuric ion has no effect on a polyalanine monolayer but affects both polylysine and polyglutamic acid in a manner similar to the proteins. This indicates that the mercuric ion interacts with the ionizable carboxyl and amino groups on the protein side chains, possibly forming some type of cross-link. The suggestion that mercuric ion interacts with carboxyl and amino groups on proteins in addition to sulfhydryl groups has been made previously [7].

If lipid molecules are incorporated in protein monolayers, an unusual effect occurs [8]. As the mixed film is compressed, the viscosity at first increases normally but then goes through a maximum and decreases with further increase of surface pressure, the behavior being reversible. This is shown in Fig. 3 for mixed monolayers of polyalanine and lecithin of

different compositions. The abnormal behavior shows that the structure of the monolayer is changing as it is compressed. Evidently the lipid molecules cause breaking of hydrogen bonds between the protein chains. This could occur simply by the lipid molecules sterically preventing protein chains from coming together. At high areas per molecule, this effect will not be very great because of the comparatively low surface density of lipid molecules. However, as the film is compressed, the effect becomes greater due to the relatively greater field of influence of the lipid molecules. In addition to the steric effect, lipid molecules may also break hydrogen bonds between protein chains by increasing the net surface charge or by forming hydrogen bonds with the protein.

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