The thickness of such a molecule from the present study would be 18.1 Å. Bull and Cooper[10] from viscosity and diffusion data estimated the thickness of the β-lactoglobulin molecule to be 20 Å. Likewise from the present study we conclude that the area of the top face of the duplex molecule would be $\frac{1}{2} \times 5800$ or 2900 sq. Å.

Summary

1. The force–area curve for β-lactoglobulin has been investigated for films of the protein spread on 35% ammonium sulfate solution. A Wilhelmy balance has been used to record the film pressures.

2. The β-lactoglobulin film is gaseous at low film pressures. The molecular weight of the β-lactoglobulin in the spread film has been estimated to be about 44,000. It is concluded that the β-lactoglobulin molecules do not associate or dissociate on the surface.

3. It has been found that the area of the gaseous, uncompressed film of β-lactoglobulin is approximately 1.2 sq. meters per milligram of protein from which it is concluded that there is extensive orientation of the side chain residues in the uncompressed state.

4. The film pressure and the corresponding film area has been determined at the point of minimum compressibility. At this point the β-lactoglobulin film occupies 0.83 sq. meter per milligram of protein. The area per molecule and the average area per amino acid residue have been calculated.

5. A tentative attempt has been made to arrive at the molecular dimensions of the native β-lactoglobulin molecule. It is concluded that the data from spread films of this protein are compatible with a duplex structure whose dimensions are reported.

(10) Bull and Cooper, *Am. Assoc. Adv. Sci. Pub.* No. **21**, 150 (1943).

CHICAGO, ILLINOIS RECEIVED MAY 27, 1944

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, NORTHWESTERN UNIVERSITY MEDICAL SCHOOL]

Mixed Monolayers of Egg Albumin and Lauryl Sulfate[1]

BY HENRY B. BULL

Bull and Neurath[2] were able to show that sulfated alkyl detergents are potent denaturing agents for proteins. This observation has since been elaborated on by several workers.[3,4,5,6,7] The sulfated detergents are notable for their surface activity and it appeared profitable to investigate the action of the detergents on spread monolayers of protein. The protein selected was egg albumin and the sulfated detergent was sodium lauryl sulfate (abbreviated NaLS in what is to follow).

Experimental.—The egg albumin was prepared from fresh hen's eggs by the method of Kekwick and Cannan.[8] It was recrystallized three times and dialyzed against distilled water until sulfate free. The NaLS was of a highly purified grade supplied by the courtesy of the Fine Chemicals Division of the E. I. du Pont and Co., Inc. Thirty five per cent. ammonium sulfate solution was used as the underlying solution and the same spreading technique was employed as described in a previous paper.[9] A Wilhelmy balance registered the film pressure.

A solution containing 0.250 mg. of NaLS per cc. and another solution containing 0.330 mg. of egg albumin per cc. were prepared. These two solutions were mixed in a series of relative concentrations which extended from pure protein to pure NaLS. For example, in the first of a series of 11 test-tubes were placed 10 cc. of the protein solution and no NaLS. The second tube contained 9 cc. of the protein solution and 1 cc. of the NaLS solution. The last tube of the series contained 10 cc. of the NaLS solution and no protein solution. These solutions were allowed to remain overnight at room temperature and then spread on 35% ammonium sulfate solution and the force–area curves determined. No control of the *p*H of the detergent protein mixtures was attempted. The protein was at its isoelectric point and the solutions of the pure sulfated detergents had a *p*H of 6.9. The 35% ammonium sulfate solution had an apparent *p*H of 3.1 as obtained with a glass electrode.

Results.—Before the results for the mixed films are reported it would be well to describe some of the properties of the pure NaLS films.

NaLS does not form a stable spread film on pure water and indeed it is not until the concentration of the underlying ammonium sulfate solution is increased up to about 27% that a stable spread film of NaLS can be obtained and even at this concentration of ammonium sulfate the spread film goes into solution in the ammonium sulfate at a film pressure of about 7 dynes per cm. At 35% ammonium sulfate in the underlying solution the spread film of NaLS is stable up to high film pressures. It was, therefore, decided to confine our attention in this study to spread films of NaLS on 35% ammonium sulfate solutions.

Figure 1 shows force–area curves of NaLS films on such a solution.

It will be noted in Fig. 1 that the NaLS can exhibit two force–area curves. The occasion for these two curves has not as yet been established. The films which give the results indicated by the half filled circles reached "equilibrium" surface

(1) Presented at the Symposium on Surface Active Agents and their Application to Biological Systems held by the Division of Physical Chemistry of the American Chemical Society at Cleveland, Ohio, April 4, 1944.

(2) Bull and Neurath, *J. Biol. Chem.*, **118**, 163 (1937).

(3) Anson, *Science*, **90**, 256 (1939).

(4) Miller and Anderson, *J. Biol. Chem.*, **144**, 475 (1942).

(5) Shock and Fogelson, *Proc. Soc. Exptl. Biol. Med.*, **50**, 304 (1942).

(6) Neurath and Putnam, *J. Biol. Chem.*, **150**, 263 (1943).

(7) Lundgren, Elam and O'Connell, *ibid.*, **149**, 183 (1943).

(8) Kekwick and Cannan, *Biochem. J.*, **30**, 227 (1936).

(9) Bull, THIS JOURNAL, **67**, 4 (1945).

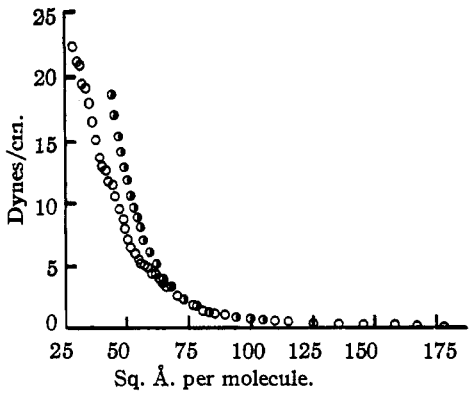

Fig. 1.—Force-area curve for NaLS spread on 35% ammonium sulfate solution.

tension almost immediately in response to a decrease in the film area while the films which are represented by the open circles were very sluggish in attaining "equilibrium." The suspicion cannot be avoided that the smaller areas exhibited by the sluggish films are due to a partial solution of the films into the underlying ammonium sulfate solution. Inasmuch as the larger areas (half filled circles) were obtained more frequently and areas of several films of this type were in close agreement, this type of film has been accepted as the standard and all calculations reported in this paper have been based on this type of film.

Figure 2 shows the areas in square meters per milligram of the mixtures of protein and NaLS plotted against the weight fraction of NaLS at 2.5 and at 15 dynes per cm. film pressure.

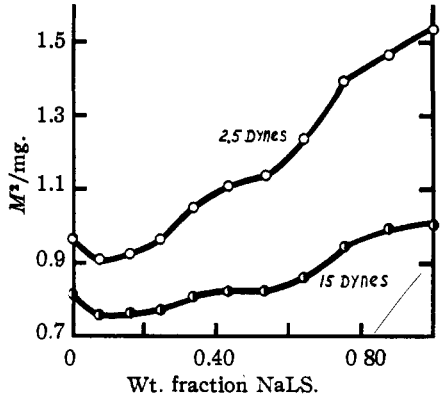

Fig. 2.—Film areas of mixtures of NaLS and protein in square meters per milligram plotted against the weight fraction of NaLS at 2.5 and 15 dynes per cm.

Discussion.—The results shown in Fig. 2 indicate extensive and complicated interaction between egg albumin and NaLS. The evidence for this interaction becomes somewhat clearer when we calculate the partial milligram surface area of the two components by the method of intercepting tangents as described by Lewis and Randall.[10] The partial milligram area of NaLS in square meters is plotted in Fig. 3 against the weight fraction of NaLS both at 2.5 and at 15 dynes per cm. while in Fig. 4 the partial milligram area in square meters for the protein is plotted against the weight fraction of protein.

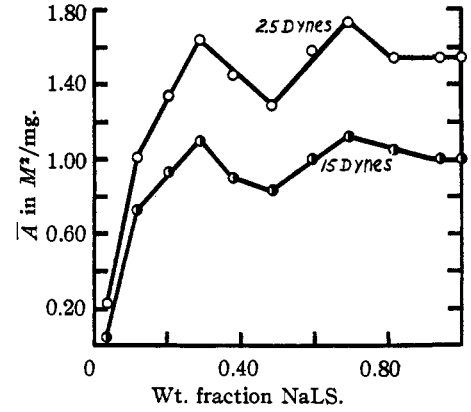

Fig. 3.—Partial milligram area of NaLS in square meters plotted as a function of the weight fraction of the NaLS at 2.5 and 15 dynes per cm.

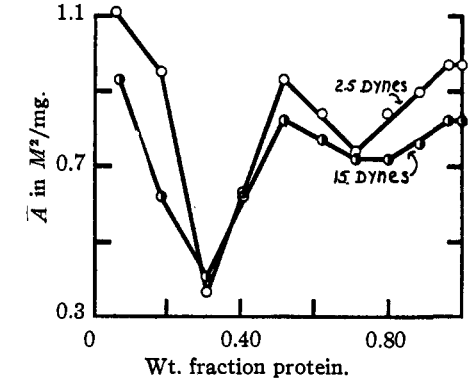

Fig. 4.—Partial milligram area of egg albumin in square meters plotted as a function of the weight fraction of egg albumin at 2.5 and 15 dynes per cm.

The first point to note from Figs. 3 and 4 is that the partial milligram areas at 2.5 and at 15 dynes per cm. vary in the same manner with weight fraction both for the protein and for NaLS. This indicates that neither component of the mixture is forced out of the film as the film pressure is increased from 2.5 to 15 dynes per cm.

It is evident from Fig. 3 that at low weight fractions of NaLS little or none of the NaLS is in the surface film; presumably it is adsorbed beneath the film. As the concentration of the NaLS is increased it begins to penetrate the film until at a weight fraction of about 0.2 the addition of NaLS increases the film area to about the same extent as it does in a pure film of NaLS.

The addition of protein to a pure NaLS film (Fig. 4) is followed by a rapid reduction of the partial area of the protein up to a protein weight fraction of about 0.3. Beyond this weight fraction the partial area increases until at a weight

(10) Lewis and Randall, "Thermodynamics," McGraw-Hill Book Co., Inc., New York, N. Y., 1923.

fraction of about 0.5 the partial area is about equal to that of the pure protein.

As a first approximation it would appear safe to assume that where the film area of the mixed film is less than the sum of the areas of the two components separately, one of the components has been displaced from the film and probably adsorbed beneath it. We have already noted this displacement is not a function of the film pressure. Provided the above assumption is admitted, we can calculate the per cent. of the area of the protein film which is covered by NaLS. The area occupied by the protein in the mixed film is evidently equal to the weight fraction of the protein multiplied by the area occupied by the pure protein film. The decrease in area due to the displacement of the NaLS from the film is equal to the difference between the measured area of the film of a given mixture and the sum of the areas of the individual components. When we multiply this difference by 100 and divide by the area occupied by the protein, we have the per cent. of the area of the protein in the film which is covered by NaLS. Figure 5 shows these percentages plotted against the weight fraction of NaLS.

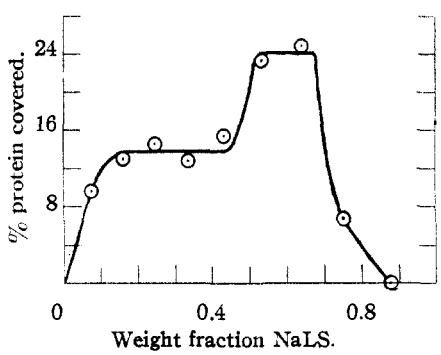

Fig. 5.—Percentage coverage of the protein film by NaLS as a function of the weight fraction of NaLS.

Figure 5 indicates three distinct steps in the interaction between protein and NaLS as the weight fraction of NaLS is increased. The first step is complete at about a weight fraction of 0.18 and represents a coverage of about 14%. The second step is complete at a weight fraction of NaLS of about 0.5 and involves a coverage of about 25%. The next step starts at a weight fraction of NaLS of about 0.65 and is complete at a weight fraction of about 0.85. The third step can be reasonably interpreted as involving a penetration of the protein film by the NaLS adsorbed beneath it. In this region the sum of the areas of the protein and NaLS separately approaches the measured area of the mixed film.

There is still another way of looking at these data. We know the total number of molecules of NaLS in any given mixture and the area which they should occupy. We can also calculate from this information and from the assumptions outlined in the previous paragraph the number of NaLS molecules not in the surface film and which are bound to the protein molecule. The results of these calculations are shown in Fig. 6.

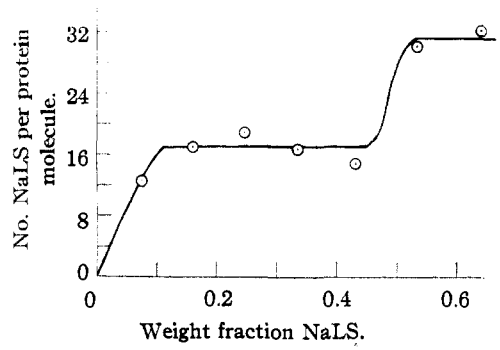

Fig. 6.—Number of NaLS molecules bound underneath the protein film per egg albumin molecule as a function of the weight fraction of NaLS.

The results shown in Fig. 6 indicate the existance of two well-defined protein–egg albumin complexes. The first complex contains about 17 NaLS molecules per molecule of egg albumin while the second complex has about 32 NaLS molecules per molecule of egg albumin.

Acknowedgment.—It is a pleasure to acknowledge the financial assistance of the Abbott Research Fund of Northwestern University which permitted the employment of technical help, Miss Lorraine Schmidt.

Summary

1. Force–area curves for films of sodium lauryl sulfate spread on 35% ammonium sulfate have been reported.

2. The film area of mixtures of egg albumin and sodium lauryl sulfate spread on 35% ammonium sulfate have been studied as a function of the weight fractions of the two components.

3. The partial milligram area in square meters has been calculated for both egg albumin and sodium lauryl sulfate.

4. The number of molecules of sodium lauryl sulfate bound per molecule of egg albumin has been calculated. Evidence is advanced for complex formation between egg albumin and sodium lauryl sulfate.

CHICAGO, ILL. RECEIVED MAY 27, 1944