the time which elapses between the spreading and the start of the compression up to an elapsed time of twenty minutes.

3. The egg albumin film is "gaseous" at low film pressures. The molecular weight of the egg albumin in the spread film has been estimated to be about 44,000. It is concluded that the egg albumin molecule probably does not dissociate on the surface.

4. It has been found that the area of the gaseous, uncompressed film of egg albumin is 0.97 sq. meter per milligram of protein from which it is concluded that there is extensive orientation of the side chain residues in the uncompressed state.

5. The film pressure and the corresponding film area have been determined at the point of minimum compressibility. At this point the egg albumin film occupies 0.82 square meter per milligram. The area per egg albumin molecule and the area per residue have been calculated.

6. It is concluded that this study is in keeping with the theory that native protein molecules of the egg albumin type are laminated structures.

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

Monolayers of β-Lactoglobulin on Concentrated Salt Solutions

BY HENRY B. BULL

In a previous paper it has been shown that egg albumin spreads in surface films on 35% ammonium sulfate solutions completely and rapidly.[1] It was also shown that a Wilhelmy balance registers the film pressure with a precision which cannot be attained except with the most carefully and expensively made torsion-float type of film balance. It was decided to employ these improvements in technique in a study of the force–area measurements of spread films of β-lactoglobulin.

Experimental.—The β-lactoglobulin was prepared from fresh, raw, whole milk according to a private communication from Dr. A. H. Palmer. It formed large plate-like crystals which were recrystallized several times. The spreading technique employed was the same as that previously described for egg albumin.[1] The concentration of the β-lactoglobulin solution was 0.0269%. The solution upon which the protein was spread was 35% ammonium sulfate. Particular attention was paid to the purity of the ammonium sulfate solutions; previous to use they were treated with activated charcoal to remove surface active impurities.

Results.—Figure 1 shows a force–area curve for a spread film of β-lactoglobulin on 35% ammonium sulfate.

The time between protein spreading and the start of compression was two minutes. The film was compressed slowly and with ample time for the attainment of equilibrium surface pressures.

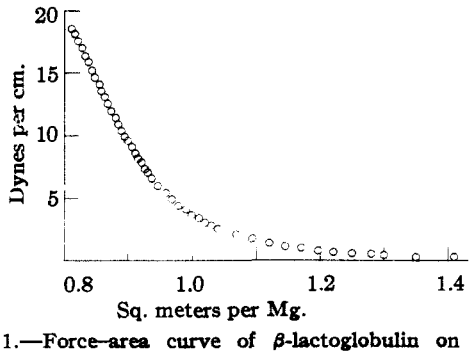

Fig. 1.—Force–area curve of β-lactoglobulin on 35% ammonium sulfate.

Discussion.—When the area in square meters per milligram is multiplied by the surface tension lowering in dynes per centimeter at pressures below about 0.5 dyne per centimeter pressure, a straight line is obtained. This is shown in Fig. 2.

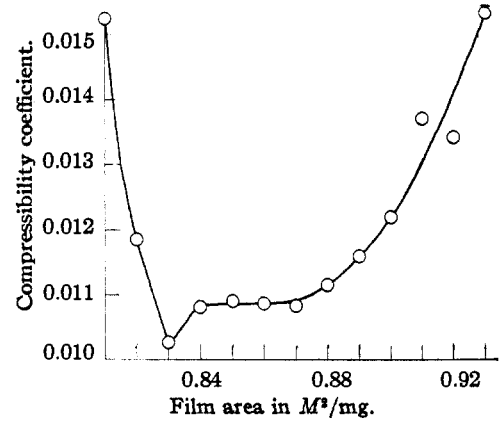

Fig. 2.—Film pressures multiplied by the corresponding film area and plotted against the film pressure.

The results in Fig. 2 illustrate the typical behavior of a so-called "gaseous" film and as we have pointed out in a previous paper[1] the ideal gas laws in 2 dimensions should apply at zero pressure.

The best straight line through the points in Fig. 2, was calculated by the method of least squares. The resulting equation is

$$FA = 1.21F + 0.0555 \qquad (1)$$

The extrapolated value of FA at zero film pressure is, accordingly, 0.0555 erg, which corresponds to a molecular weight of about 44,000. The molecular weight of β-lactoglobulin from equilibrium ultracentrifugation is 38,000 while from rate ultracentrifugation it is 41,500.[2]

(1) Bull, THIS JOURNAL, 67, 4 (1945).

(2) Svedberg and Pedersen, "The Ultracentrifuge," Oxford University Press, New York, N. Y., 1940.

Brand and Kassell[3] from amino acid analysis conclude that the molecular weight of this protein is 42,000. It is clear that provided the force of attraction between the spread β-lactoglobulin molecules on the surface is negligible in the low pressure region, the β-lactoglobulin molecules do not dissociate when spread in a monolayer.

From equation 1 we conclude that the area of the gaseous spread β-lactoglobulin molecules is approximately 1.2 square meters per milligram.

Turning now to a consideration of the spread film in its compressed state, we plot the coefficient of compressibility of the film against its area in square meters per milligram. This plot is shown in Fig. 3.

It is evident that the coefficient of compressibility shows a sharp minimum at 0.83 square meters per milligram which corresponds to a film pressure of 16.7 dynes per centimeter. The area of 0.83 square meter per milligram of protein is believed to be the area of maximum compression without collapse of the film. If we accept the value of Brand and Kassell for the molecular weight of β-lactoglobulin, the area occupied by a molecule in a compressed spread film is 5,800 square Å. Brand and Kassell estimate that β-lactoglobulin molecule contains 364 amino acid residues from which the apparent average area per residue is 15.9 square Å. In a previous paper we have discussed our reasons for believing that the amino acid residue side chains alternate above and below the plane of the peptide chains and accordingly the true average area per residue is twice 15.9 sq. Å. or 31.8 sq. Å.

If the spread film is dehydrated in its compressed state and has the density which the protein has in bulk, its thickness is 9.05 Å.

The approximate area of the gaseous film is 1.21 sq. meters per milligram. While this represents an appreciable spreading apart of the peptide chains from the area of 0.83 sq. meters per milligram of the compressed film, it is still not nearly as much as would be expected if the molecules were completely expanded with the side chain residues lying flat on the surface as the area of such an expanded film should be about 1.6 sq. meters per milligram. We can conclude that while there is extensive orientation of the side chain residues in a gaseous, uncompressed β-lactoglobulin film, the orientation is not so good as it is in a gaseous egg albumin film where the area is 0.97 sq. meter per milligram.

An attempt was made to discover whether or not there was a progressive expansion of the gaseous β-lactoglobulin molecules on the surface. The results of this attempt were rather ambiguous. It is true that there is an apparent expansion with time but the difficulty of protecting the film from surface contaminants was so great that it may well be that this apparent expansion was due to contamination of the surface by surface

(3) Brand and Kassell, *J. Biol. Chem.*, **145**, 365 (1942).

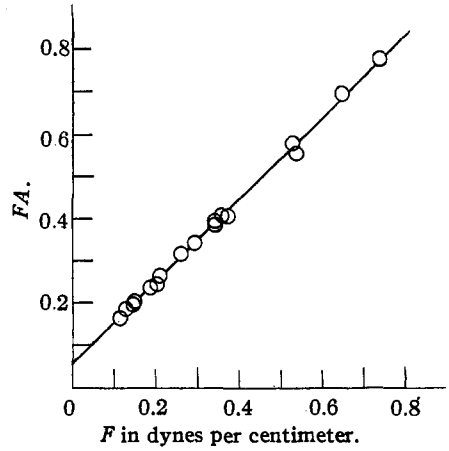

Fig. 3.—Coefficient of compressibility plotted against the film area in square meters per milligram.

active impurities. It was noted, for example, that the more complete the protection against impurities, the smaller was the apparent expansion of the gaseous molecules with time. We are unable, therefore, to draw any definite conclusions regarding this point.

Astbury,[4] Pauling,[5] Dervichian,[6] Boyes-Watson and Perutz[7] and Palmer[8] have suggested that native protein molecules of the β-lactoglobulin type are layer-like structures. It is believed, as Dervichian in particular has pointed out, that studies on spread films of proteins strongly substantiate this conclusion regarding the layer structure of proteins. It is not yet clear how many layers of peptide chains exist in a given native protein molecule. Boyes-Watson and Perutz appear to have fairly unambiguous X-ray diffraction data on methemoglobin crystals which indicate that this protein has four such layers per molecule. There is some reason to believe, however, that this number might not be correct for egg albumin and for β-lactoglobulin. For example, the hemoglobin molecule readily splits into halves[9] in urea indicating a hydrophilic plane of cleavage in this molecule. With Dervichian, the writer believes that a 2 layer structure for egg albumin and for β-lactoglobulin is in keeping with everything we know about these proteins and since this represents a simpler assumption than a four layer structure, a structure consisting of 2 parallel layers of peptide chains will be tentatively accepted for these proteins. In such a structure the outer faces would be hydrophilic and would contain all or practically all the charged groups. The inner planes would contain the hydrophobic groups. Spreading on the surface would involve the opening of the molecule in the same manner that a book is opened.

(4) Astbury, *Nature*, **137**, 803 (1936).
(5) Pauling, THIS JOURNAL, **62**, 2643 (1940).
(6) Dervichian, *J. Chem. Phys.*, **11**, 236 (1943).
(7) Boyes-Watson and Perutz, *Nature*, **151**, 714 (1943).
(8) Palmer, *J. Phys. Chem.*, **48**, 12 (1944).
(9) Steinhardt, *J. Biol. Chem.*, **138**, 543 (1938).

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).

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[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).