The β-lactoglobulin used in the previous film studies had been recrystallized by the addition of NaOH to the crystals with subsequent neutralization with hydrochloric acid. Bull and Currie[2] have shown that this technique leads to a partially aggregated material. When spread in a surface film, this aggregated protein does not dissociate either because of the changes introduced by the use of alkali in the preparation of the protein or more probably because cupric ions were present in the ammonium sulfate solutions; the importance of cupric ions was not realized at the time these earlier experiments were done.

Since as a result of osmotic pressure measurements[2] the molecular weight of β-lactoglobulin has had to be revised from 42,000 to 35,050, it is necessary also to revise the calculated surface area per molecule at minimum compressibility from 5,800 to 4,850 sq. Å. This revision of the molecular weight does not of course change the calculated area per amino acid residue nor the thickness of the surface film at the point of minimum compressibility.

In view of the pronounced influence of cupric ions on gaseous films of β-lactoglobulin the effect of such ions on gaseous film of egg albumin was studied. A film molecular weight of about 44,000 had been reported for this protein.[3] A series of solutions containing 20% ammonium sulfate were prepared. Cupric sulfate was added to these solutions so that the maximal concentration of cupric sulfate was 4.8×10^{-4} molar. The film molecular weight of egg albumin was found to be independent of the concentration of cupric ions and an average of eleven determinations gave a film molecular weight of 44,500 which is in good agreement with the previously reported value for this protein.

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

Summary

1. Gaseous films of β-lactoglobulin spread on ammonium sulfate solutions have been investigated in some detail and the various factors which might influence the behavior of such films have been studied.

2. It is found that β-lactoglobulin when spread in surface films dissociates into two surface active fragments whose average molecular weight is close to 17,000 and whose average area is 1.25 sq. meters per milligram.

3. If cupric sulfate be added to the ammonium sulfate solutions to yield concentrations equal to or greater than 2.5×10^{-4} molar, the average surface molecular weight becomes 34,300 and the average area of the gaseous molecules is 1.40 sq. meters per milligram of β-lactoglobulin.

CHICAGO, ILLINOIS RECEIVED DECEMBER 19, 1945

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

Mixed Monolayers of β-Lactoglobulin and Lauryl Sulfate

BY HENRY B. BULL

In a previous paper[1] it was shown that egg albumin and lauryl sulfate form definite complexes the existence of which can be detected by force-area measurements on mixed surface films of protein and detergent. The present paper deals with such a study on surface films of mixtures of sodium lauryl sulfate (abbreviated NaLS) and β-lactoglobulin.

Experimental

The β-lactoglobulin was prepared from fresh, whole raw cows' milk by the method described by Bull and Currie.[2] The protein was recrystallized several times from 0.07 *M* sodium chloride by dialysis against distilled water. The NaLS was a highly purified grade supplied through the courtesy of the Fine Chemical Division of E. I. du Pont de Nemours and Company, Inc.

A solution containing 0.35 mg. of β-lactoglobulin per cc. and one containing 0.25 mg. of NaLS were prepared. These two solutions were mixed in a series of relative concentrations which extended from pure protein to pure NaLS. These solutions were allowed to remain overnight and then spread on 35 per cent. ammonium sulfate solutions. A Wilhelmy balance was used to register the film pressure. The balance was set at a film pressure of 10 dynes per centimeter and the surface films compressed until this pressure was reached and the film areas noted. Determinations were made in duplicate.

A series of ammonium sulfate solutions were prepared containing respectively 5, 10, 15, 20, 30 and 35% salt. Pure

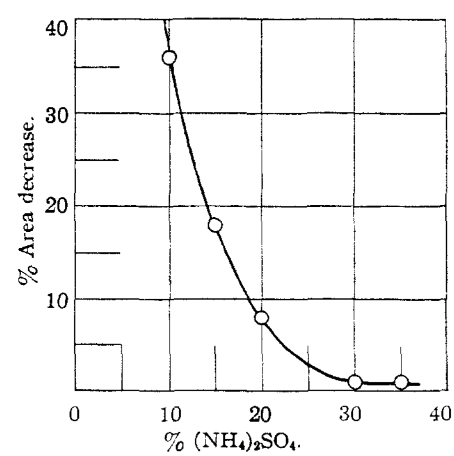

Fig. 1.—Per cent. decrease in area of films of NaLS at 10 dynes per centimeter pressure in ten minutes as a function of ammonium sulfate concentration of substrate solution.

(1) Bull, THIS JOURNAL, **67**, 10 (1945).

(2) Bull and Currie, *ibid.*, **68**, 742 (1946).

NaLS solutions were spread on these solutions and the per cent. decrease of the film area in ten minutes at 10 dynes per centimeter pressure noted. The results of this study are shown in Fig. 1. Evidently, above a concentration of about 30% ammonium sulfate spread films of NaLS are stable at 10 dynes film pressure.

Results

Figure 2 shows the plot of the area of the spread films at 10 dynes per centimeter film pressure per milligram of detergent plus protein against the weight fraction of detergent.

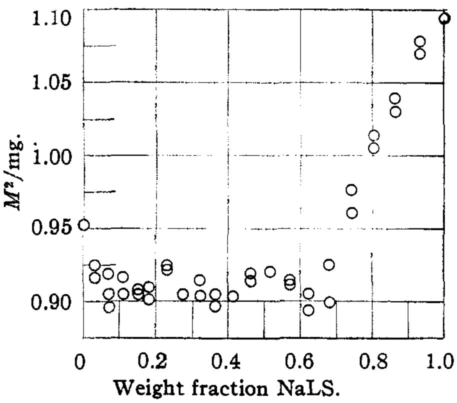

Fig. 2.—Area of the spread film in square meters per milligram at 10 dynes per centimeter of film pressure against the weight fraction of NaLS.

Discussion

Treating the results of this study in the same way as was described for mixed films of egg albumin and detergent,[1] we can calculate the number of detergent molecules bound per molecule of protein. The results of these calculations based on a molecular weight of 35,000 for β-lactoglobulin[2] are shown in Fig. 3.

There is an inflection in the curve (Fig. 3) at about 8 molecules of NaLS per molecule of protein molecule and the curve shows a decided break at about 40 detergent molecules per protein molecule. On the basis of a molecular weight of 35,000 the sum of the positive groups in β-lactoglobulin (histidine, lysine, and arginine) is about 37.[3] It seems probable that the curve shown in Fig. 3 is in reality a titration curve for the basic groups by the anionic detergent.

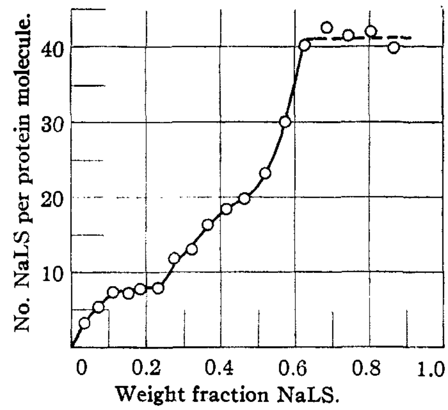

Fig. 3.—Number of NaLS molecules bound per protein molecule as a function of the weight fraction of NaLS.

Summary

1. The film areas of mixtures of β-lactoglobulin and sodium lauryl sulfate spread on 35% $(NH_4)_2SO_4$ and at 10 dynes per centimeter film pressure have been reported.

2. The number of molecules of sodium lauryl sulfate bound per β-lactoglobulin molecule has been calculated and plotted as a function of the concentration of detergent.

(3) Brand, Saidel, Goldwater, Kassel and Ryan, This Journal, **67**, 1524 (1945).

Chicago, Illinois Received December 19, 1945

[Contribution from Mellon Institute of Industrial Research]

The Structure of Ethylene Polysulfides[1,2]

By Harry E. Westlake, Jr., M. Glenn Mayberry, Maurice H. Whitlock, James R. West and George J. Haddad

Armstrong, Little and Doak[3] have isolated and identified as monosulfides some of the light products from the reaction of sulfur with certain higher olefins and have described the formation of unidentified polymeric compounds. Earlier Friedman[4] obtained sulfides from the reaction of sulfur with butylene. Ethylene, the simplest unsaturated compound, seems to have been largely neglected. Victor Meyer[5] mentioned its reaction with sulfur at 300° to give a trace of thiophene, and Jones and Reid[6] found that hydrogen sulfide and ethyl mercaptan were formed at 325°. Meyer and Hohenemser[7] reported no reaction at 140°. We have found, however, that extensive reaction occurs at temperatures above the melting point of sulfur.

(1) Contribution from the multiple industrial fellowship sustained at Mellon Institute by the Texas Gulf Sulphur Company, New York, N. Y.

(2) Presented before the Division of Organic Chemistry at the 108th meeting of the American Chemical Society, New York, N. Y., Sept. 14, 1944.

(3) Armstrong, Little and Doak, *Ind. Eng. Chem.*, **36**, 628 (1944); *Rubber Chem. Tech.*, **17**, 788 (1944).

(4) Friedman, *Petroleum*, **11**, 693 (1916).

(5) Victor Meyer, "Die Thiophengruppe," Braunschweig, Germany, 1888, p. 16.

(6) Jones and Reid, This Journal, **60**, 2452 (1938).

(7) Meyer and Hohenemser, *Helv. Chim. Acta*, **18**, 1061 (1935).