urea solutions. In view of the small concentration of protein used, we regard this interpretation as unlikely. The apparent increase of the molecular weights in 7 and in 8 *M* urea is undoubtedly due to progressive aggregation as both solutions showed a degree of turbidity which was not present in the other urea concentrations used.

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We also wish to thank Drs. W. Heller and H. B. Klevens for their kind permission to use their unpublished results on light scattering.

Summary

1. Twenty osmotic pressure measurements on solutions of β-lactoglobulin in 0.5 *M* sodium chloride have been reported. A molecular weight of 35,050 with a standard deviation of the mean of 144 has been calculated.

2. The osmotic pressure of β-lactoglobulin solutions in several concentrations of urea have been measured. It is concluded that urea probably dissociates β-lactoglobulin into two fragments.

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

Monolayers of β-Lactoglobulin. II. Film Molecular Weight

BY HENRY B. BULL

In a previous paper[1] it was shown that β-lactoglobulin prepared from fresh, raw whole milk forms a gaseous monolayer on the surface of concentrated ammonium sulfate solutions and from the application of the gas laws in two dimensions, the molecular weight along with the area of the gaseous molecules was calculated. The film molecular weight reported for β-lactoglobulin was about 44,000 and the area of the gaseous film was 1.21 sq. meters per milligram of protein. Since that time, the molecular weight of β-lactoglobulin has been determined by osmotic pressure measurements[2] and found to be 35,050 with a standard deviation of the mean of 144. In view of this discrepancy between the results of these two techniques, it was decided to reinvestigate in greater detail gaseous spread films of β-lactoglobulin on concentrated ammonium sulfate solutions. It has now been found possible to reconcile the results from surface film technique and from osmotic pressure measurements. These studies are reported in this paper.

Experimental

The β-lactoglobulin was prepared from fresh, raw whole milk by the technique described by Bull and Currie.[2] The protein was recrystallized several times by dialyzing a solution of the protein in 0.07 *M* sodium chloride against water. The protein crystals were dissolved in 1 *M* sodium chloride and enough water added to make the solution 0.5 *M* in respect to sodium chloride. The protein concentration was determined with a dipping refractometer (Zeiss). The mother solutions were diluted with water so that one Blodgett pipet would deliver between 0.02 and 0.03 mg. of β-lactoglobulin to the surface of the ammonium sulfate solutions.

A Wilhelmy balance has been used to measure the surface pressures. Two thin microscope cover glasses each 6 cm. wide were suspended from one arm of the analytical balance. This gives a total length of surface of 24 cm. and, accordingly, one milligram of weight was equivalent to 0.0409 dyne per centimeter film pressure. It was noted that "tears" of ammonium sulfate solution tended to form on the Wilhelmy slides when the slides were placed in the clean ammonium sulfate solution surface. This led to confusion in regard to the initial base line weight in the absence of protein since as soon as protein was added to the surface these "tears" drained off the slide and, accordingly, the weight of the suspended glass slides changed. The technique adopted to avoid this difficulty was to spread protein on the surface of the ammonium sulfate solution with the Wilhelmy slides in place and then to sweep the surface of the ammonium sulfate solutions repeatedly with a movable barrier. This gave an unambiguous base line for the weight of the slides in the absence of protein.

Previous to use, the ammonium sulfate solutions were treated with activated charcoal to remove surface active impurities.

In all cases, the film pressures in dynes per centimeter have been multiplied by the corresponding film areas in sq. meters per milligram and those values plotted against the film pressures. These plots included eight or nine points extending from about 0.1 dyne per centimeter up to about 0.5 dyne per centimeter. The best straight line was drawn through these points and the slope and intercept on the *y*-axis determined. The slope of the line is equal to the area occupied by the gaseous molecules in sq. meters per milligram. For one mole of substance in the surface film, the intercept at 25° should equal 24.6×10^3 ergs when the pressure is expressed in dynes per centimeter and the area in sq. meters per milligram. Accordingly the film molecular weight is equal to 24.6×10^3 divided by the value of the intercept.

Results

β-Lactoglobulin was spread on 20% ammonium sulfate and the influence of time on the properties of the gaseous surface film studied. The film was compressed within one minute after spreading and the pressure–area curve measured. The film was then expanded and recompressed at the end of fifteen minutes. It was re-expanded and recompressed and re-expanded again at the end of thirty minutes, at the end of forty-five minutes and at the end of four hours. During this time the base line for the weight of the slide had

(1) Bull, THIS JOURNAL, **67**, 8 (1945).

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

changed (increased) appreciably and, accordingly, it was necessary to add to each starting weight at full expansion a correction factor the value of which was determined by calculating the weight which had to be added to give a linear relation when the film pressure multiplied by the film area is plotted against the film pressure. Table I shows the results of these experiments.

TABLE I

SURFACE MOLECULAR WEIGHTS AND AREAS OF GASEOUS FILMS OF β-LACTOGLOBULIN AS A FUNCTION OF TIME THE SURFACE FILM HAD REMAINED ON THE SURFACE OF A 20% AMMONIUM SULFATE SOLUTION

Time between spreading and compression	Area gaseous molecules in sq. meters per mg.	Mol. wt.
1 min.	1.17	17,600
15	1.25	14,700
30	1.27	18,100
45	1.24	17,600
4 hr.	1.36	16,200

From Table I it can be seen that there is no significant change of the film molecular weight with time. The gaseous protein molecules do, however, appear to undergo a very slow expansion on the surface.

The influence of the ammonium sulfate concentration on the gaseous β-lactoglobulin was investigated and these results are shown in Table II.

TABLE II

FILM MOLECULAR WEIGHTS AND AREAS OF GASEOUS β-LACTOGLOBULIN AS A FUNCTION OF THE AMMONIUM SULFATE CONCENTRATION

Concn. $(NH_4)_2SO_4$, %	Area in sq. meters per mg.	Mol. wt.
10	1.17	14,500
20	1.19	16,400
30	1.40	20,500
40	1.45	16,400

The film molecular weight of β-lactoglobulin is independent of the concentration of ammonium sulfate. However, there is a tendency for the gaseous β-lactoglobulin molecules to expand on the higher concentrations of ammonium sulfate.

It was found that the addition of ammonium hydroxide to produce a concentration as high as 0.03 *N* in the ammonium sulfate solution was without influence on the film molecular weight or on the area of the gaseous molecules. Likewise an excess of sulfate (produced by the addition of sulfuric acid to the ammonium sulfate solution) did not affect the properties of the gaseous surface film.

For various reasons it was suspected that heavy metal cations might have a pronounced effect upon the gaseous films of β-lactoglobulin. In previous measurements[1] the trough had in it an outlet which was stoppered by a brass screw. In spite of careful coating of the trough with wax, it was noted that this screw had become eroded. At the beginning of the present study this screw was removed and replaced by a non-metallic plug so that all experiments reported in the present paper were done under conditions which excluded contamination by heavy metal cations. A series of solutions of ammonium sulfate containing 23% of the salt were prepared. Increasing amounts of cupric sulfate were added to these solutions and the β-lactoglobulin solutions spread on these ammonium sulfate solutions and the properties of the gaseous protein films studied as a function of the cupric sulfate concentration. These results are shown in Table III.

TABLE III

INFLUENCE OF CUPRIC SULFATE ON THE MOLECULAR WEIGHT AND AREA OF GASEOUS FILMS OF β-LACTOGLOBULIN SPREAD ON 23% AMMONIUM SULFATE SOLUTIONS

Molar concn. $CuSO_4$	Area in sq. meters per mg.	Mol. wt.
0.0	1.28	16,300
1.25×10^{-4}	1.32	17,900
2.50×10^{-4}	1.36	30,800
2.50×10^{-4}	1.45	30,400
3.75×10^{-4}	1.28	32,400
3.75×10^{-4}	1.45	35,200
5.02×10^{-4}	1.37	31,600
6.27×10^{-4}	1.44	37,200
7.52×10^{-4}	1.44	32,800

Discussion

Evidently, on pure ammonium sulfate solutions and in the absence of heavy metal cations the β-lactoglobulin molecules dissociate into two surface active fragments whose average molecular weight is very nearly 17,000. In fact, 18 measurements on ammonium sulfate solutions in the absence of heavy metal cations yields a mean molecular weight of 17,100 with a standard deviation of the mean of 490. There is no way of judging whether or not these two fragments have equal molecular weights or not. Twice the film molecular weight ($2 \times 17,100$) is 34,200 which compares favorably with 35,020 from osmotic pressure measurements.[2] The average area of the gaseous molecules is 1.25 sq. meters per milligram of protein.

The average molecular weight in the presence of 2.5×10^{-4} molar cupric sulfate and up through 7.5×10^{-4} molar cupric sulfate is 34,300 and the average area of the gaseous film is 1.40 sq. meters per milligram. Evidently, in the presence of cupric ion β-lactoglobulin does not dissociate or, if it does, it recombines to form molecules whose weight is that of the original molecule. The area of the gaseous molecule is significantly higher in the presence of cupric ions. This may mean that there is a poor fit between the two fragments of the molecule and accordingly the protein occupies a larger surface area than it does when the two fragments are completely detached from each other.

In light of the osmotic pressure measurements by Bull and Currie[2] and of the present investigation, the film molecular weight of 44,000 which had been previously reported[1] can now be understood.

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

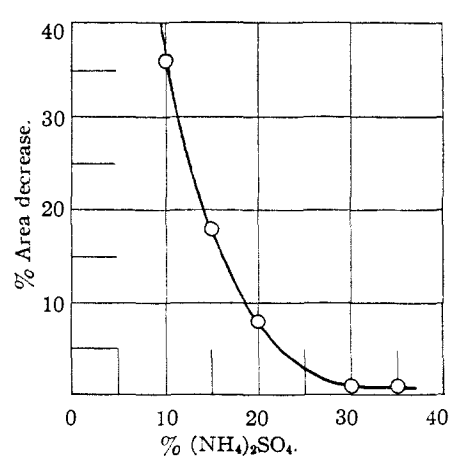

Fig. 1.—Per cent. decrease in area of films of NaLS at 10 dynes per centimeter pressure in ten minutes as a function of ammonium sulfate concentration of substrate solution.

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

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