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ADSORBED FILMS OF BOVINE SERUM ALBUMIN: TENSIONS AT AIR–WATER SURFACES AND PARAFFIN–WATER INTERFACES

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SUMMARY

Using the pendant-drop method, the interfacial tension between *n*-octadecane and aqueous solutions of bovine serum albumin reached constant values after about 30 min, and became independent of protein concentration above about 0.025%. At low ionic strength there is a minimum in the interfacial tension in the isoelectric region whereas at higher ionic strengths there is a tendency for a maximum to occur in this pH region. The addition of octadecylamine to the *n*-octadecane decreases considerably the interfacial tension against water both in the presence and absence of bovine serum albumin. The entropy change per unit area of interface goes through a sharp maximum with increasing concentration of bovine serum albumin. The compressibilities of the interfacial bovine serum albumin films as obtained from decreasing the size of the drop increases sharply at a film pressure of about 22 dynes/cm.

INTRODUCTION

There have been many studies of various kinds on protein films adsorbed at surfaces and interfaces. Several of these have dealt with the surface tension of protein solutions. The outstanding conclusion of such investigations is that the surface tension of a protein solution is a marked function of time, and equilibrium values are not attained. This ambiguity has discouraged research in this potentially interesting area.

The present work describes measurements of the surface tension at the air-BSA solution surface as well as the interfacial tensions between the BSA-solutions and *n*-octadecane using the pendant-drop method. Several experiments were done with egg albumin under similar conditions for the sake of comparison.

EXPERIMENTAL

BSA was from Armour Pharmaceutical Co. (lot No. V68802) and was passed through mixed resin column before use. Egg albumin was prepared by the method of KEKWICK AND CANNAN[1] and dialyzed exhaustively against water. The concentrations of both proteins were determined by dry weight at 105° for 24 h. Sodium acetate buffers were

Abbreviation: BSA, bovine serum albumin.

used unless otherwise stated. The *n*-octadecane was olefin-free and supplied by Humphrey Wilkinson, Inc. It was further purified by shaking with hot concentrated alkali and washing with hot water until alkali-free.

It is well known that surface tension of protein solutions changes for a long period of time. Considerable attention was given, therefore, to the choice of a proper method for the measurement of equilibrium tensions. Preliminary experiments employing the drop-weight method showed that even at a very slow rate of drop formation (30–40 min/drop) a constant value of surface tension was not reached.

The pendant-drop method[2] was found to be the most suitable for the measurement of the surface and interfacial tensions of protein solutions, there being little or no disturbance of surface during the course of the measurement. A drop of the protein solution was formed on the ground tip of a burette, the drop hanging in air or in *n*-octadecane, maintained at 30° in a water bath. The optical system of a Perkin–Elmer Model 38 electrophoresis apparatus was used to cast a sharp image of the drop

TABLE I

INTERFACIAL TENSIONS DETERMINED BY PENDANT-DROP METHOD

Interface	*Tensions in dynes per centimeter*	
	Pendant drop (our values)	*From the literature*
Water–air	71.53, 71.86 (25°)	71.97 (25°) (ref. 4)
Benzene–air	28.12 (25°)	28.23 (25°) (ref. 5)
n-Octadecane–buffer	43.97, 43.93 (30°)	—

on a photographic plate; the drop was formed where the electrophoresis cell is usually placed. The dimensions of the image of the drop were measured with a traveling microscope and the interfacial tension obtained from FORDHAM's tables[3]. The calculated tensions were accurate and reproducible to less than one dyne/cm which is sufficient for our present purposes. Values obtained by the method are shown in Table I.

Photographs were taken of a drop at varying intervals and the change of the interfacial tension followed with time. The pressure–area relation of adsorbed films of BSA at the solution *n*-octadecane interface was investigated. After 45 min–1 h a photograph of the drop was made. The drop size was decreased by withdrawing solution through the burette tip in a series of discrete steps with 2-min intervals between each step and pictures taken at the end of each interval. The differences in tension between that of the interface in the absence of protein and that with the protein present gave the film pressure of the adsorbed protein. The pictures of the drops were projected on a screen and the enlarged contour of the drop drawn and the area measured with a planimeter. The area of the drop is expressed as 4 times the area of the projected cross-section, corrected for magnification. From this area, the area of the tip of the capillary was subtracted to obtain the area of the interface.

The amount of BSA adsorbed at the *n*-octadecane–water interface was obtained by determining the adsorption of BSA on *n*-octadecane emulsions in water prepared with a sonic oscillator. The surface areas of the emulsions were estimated from turbidity measurements using a Klett-Summersen photoelectric colorimeter. The turbidities

had been previously calibrated against the surface area of emulsions as measured with a Coulter Counter; the particle size distribution was determined with the Coulter Counter and the interfacial area of the emulsion calculated therefrom.

RESULTS

Fig. 1 shows some typical data on the change of the surface and interfacial tensions of protein solutions with time. It will be noted that whereas the air–solution surface tension is not constant even after one hour, the interfacial tensions at the *n*-octadecane-solution interfaces are very nearly if not actually constant between 30 min

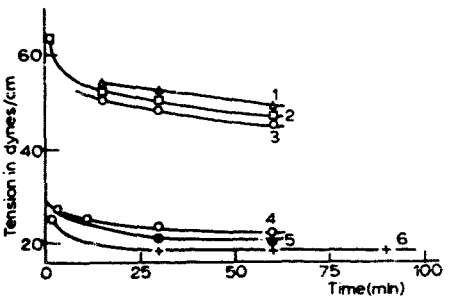

Fig. 1. Change of tensions with time. Curve 1, 0.06% egg albumin in air (pH 5.85); Curve 2, 0.06% egg albumin in air (pH 4.9); Curve 3, 0.06% BSA in air (pH 4.9); Curve 4, 0.06% egg albumin against *n*-octadecane (pH 4.2); Curve 5, 0.0018% BSA against *n*-octadecane (pH 4.17), Curve 6, 0.05% BSA against *n*-octadecane (pH 4.90). Sodium acetate buffers at 30°.

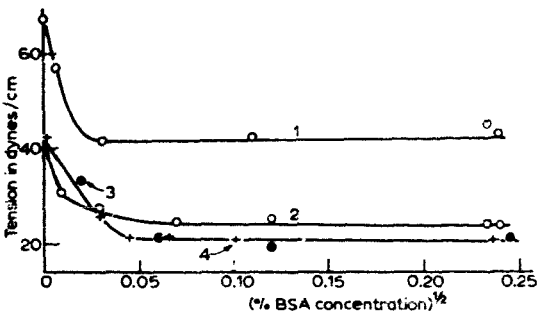

Fig. 2. Influence of BSA concentration on 1-h tensions. Curve 1, solution–air (pH 5.9) at 25°; Curve 2, *n*-octadecane-solution interface (pH 5.9) at 30°; Curve 3, *n*-octadecane-solution interface (pH 4.34) at 30°; Curve 4, *n*-octadecane-solution interface (pH 4.20) at 30°.

and 1 h. At lower protein concentrations, for example $2.5 \cdot 10^{-5}$% BSA, however, there was a decrease in the interfacial tension during the 30-min–1-h interval. The change of the interfacial tension with time appears to be independent of the pH and the ionic strength, however, the tensions fell more rapidly at 40° than at 30°.

Fig. 2 shows the influence of the BSA concentration on the 1-h values for the surface and interfacial tensions at 30°. The tensions, it will be observed, have reached constant values at a concentration as low as $1 \cdot 10^{-3}$% BSA for the air–water surfaces as well as for the paraffin–water interfaces.

TABLE II

INTERFACIAL TENSIONS BETWEEN 0.06% BSA IN 0.025 M SODIUM ACETATE BUFFERS AND *n*-OCTADECANE AT 30°

pH	2.0	3.85	4.20	4.95	5.90	6.3
Tension	21.2	20.4	20.4	18.2	23.1	19.4

The 1-h interfacial tensions between 0.06% BSA and *n*-octadecane at several pH values in 0.025 M sodium acetate buffers at 30° are shown in Table II. Only a small increase in the interfacial tensions was noticed on either side of the isoelectric point. The influence of pH on the tensions was further investigated by determining the tension for egg albumin and BSA solutions in air and of BSA solutions against *n*-octadecane using very dilute HCl or NaOH to adjust the pH and NaCl to yield the desired ionic strength. The results of these studies are shown in Fig. 3. It is to be

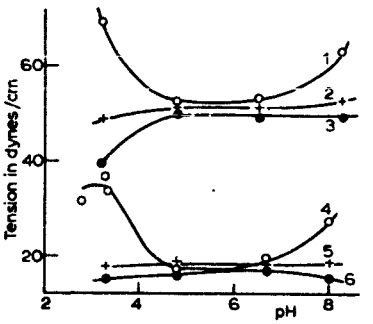

Fig. 3. Surface air-solution and interfacial tensions BSA solutions–*n*-octadecane as functions of pH, 0.06% BSA. Curve 1, air–solution, no salt; Curve 2, air–solution, 0.025 M NaCl; Curve 3, air–solution, 0.50 M NaCl; Curve 4, *n*-octadecane–solution, no salt; Curve 5, *n*-octadecane–solution, 0.025 M NaCl; Curve 6, *n*-octadecane–solution, 0.50 M NaCl.

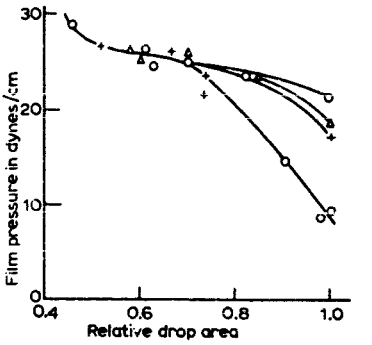

Fig. 4. Pressures of BSA films adsorbed at the solution–*n*-octadecane interface. pH 4.2, ionic strength 0.05 at 30°. Curves upper to lower: $1.8 \cdot 10^{-3}$% BSA; $9.0 \cdot 10^{-4}$% BSA; $9.0 \cdot 10^{-4}$% BSA (pH 5.90); $2.7 \cdot 10^{-4}$% BSA.

noted that in the absence of salt, the value of the tensions are significantly higher in the acid and alkaline range than they are in the pH range closer to the isoelectric point of BSA. The addition of salt, however, produces a considerable lowering of the tensions both in the acid and alkaline regions. Essentially, the same effect of ionic strength was also observed for the surface pressure of the egg albumin solutions.

Interfacial tensions between solutions of BSA against *n*-octadecane were measured at 30° and at 40° and the results are shown in Table III at pH 5.9, and ionic strength 0.05.

The interfacial tension between solutions of 0.06% BSA at pH 5.90, with an ionic strength of 0.025 and *n*-octadecane containing varying amounts of octadecylamine was measured at 30° and these results are recorded in Table IV. Also shown are the film

TABLE III

EFFECT OF TEMPERATURE ON THE INTERFACIAL TENSION OF BSA SOLUTIONS AGAINST *n*-OCTADECANE

Interfacial tensions expressed in dynes /cm. pH 5.9, ionic strength 0.05.

Percent concentration BSA	0	$1.2 \cdot 10^{-4}$	$1.8 \cdot 10^{-3}$	$8 \cdot 10^{-3}$
Tension at 30°	43.9	30.5	20.1	17.3
Tension at 40°	42.9	27.9	19.5	18.7

pressures produced by the octadecylamine as well as by the 0.06% BSA. It will be observed from Table IV that the interfacial tension of the buffer with or without BSA against the paraffin decreases considerably as the concentration of the octadecylamine in the paraffin is increased; BSA increases the film pressure even at the highest concentrations of the octadecylamine. However, the pressure due to BSA decreases with increasing concentrations of octadecylamine.

TABLE IV

EFFECT OF OCTADECYLAMINE ON THE INTERFACIAL TENSIONS AND PRESSURES BETWEEN 0.06% BSA SOLUTIONS AND *n*-OCTADECANE

Temperature 30°, pH 5.90, ionic strength 0.025 (ODA, octadecylamine).

mmoles ODA/g n-octadecane	*Tensions*		*Film pressures*	
	Without BSA	*With BSA*	*ODA*	*BSA*
0	43.9	22.2	0	21.7
0.000 25	40.3	17.1	3.6	23.2
0.000 50	31.0	15.1	12.9	15.9
0.0010	23.0	12.7	20.9	10.2
0.0020	18.4	9.6	25.5	8.8
0.0040	10.5	5.7	33.4	4.8

The pressure–area relationships of the film of BSA adsorbed at the solution–*n*-octadecane interface at various protein concentrations at pH 4.2, ionic strength 0.05 and at 30° are shown in Fig. 4. The areas are expressed as areas relative to that of the original area of the drops. The decrease in drop area has been produced by sucking the solution back into the burette in discrete steps. It will be noticed that even at the lowest concentration of BSA studied ($2.5 \cdot 10^{-5}$%) the equilibrium value of the pressure was already about 9 dynes/cm and the compression curves, therefore, give the pressure–area relations above such pressures.

DISCUSSION

The pendant-drop method for the surface and interfacial tensions of protein solutions appears to be especially suitable; the complex protein film is disturbed less during the measurement than with any of the other methods available (the capillary-rise method has to be rejected for other reasons). Constant values for the interfacial tensions for the paraffin solution interface were reached in about 30 min. A slow change of the surface tension with time was, however, observed at the air–solution surface even after 30 min. This observation is similar to those of HAUSER AND SWEARINGEN[4] using the pendant-drop method and unbuffered solutions of egg albumin. A part of this change may be due to evaporation and to surface contamination which are difficult to rule out completely at an air–solution surface. A quicker attainment of constant tensions at a paraffin–water interface is perhaps due to the reduction of the cohesion between the non-polar side chains of the protein at the oil phase thus increasing the rate at which the protein molecules relax.

The rate of change of the surface tension of the protein solutions was dependent on temperature. HAUSER AND SWEARINGEN[4] found no change of the surface tension with time for 0.001% isoionic egg albumin whereas in the present work even

$2.5 \cdot 10^{-5}$% BSA in acetate buffer exhibited a time dependence of the surface tension, the rate of change being greater at 40° than at 30°. The 1-h values for the surface tension of solutions of BSA and of egg albumin obtained by us were lower than those reported for egg albumin by the above workers, and were very near to the infinite time values which they obtained by extrapolation. In general, however, and under similar conditions our results are comparable with those of HAUSER AND SWEARINGEN.

Fig. 3 shows the remarkable influence of ionic strength on the values of tensions both at air–solution and at paraffin–solution interfaces, at pH 3 and 8; a similar influence of ionic strength on the tensions of egg-albumin solutions was also observed. It will be recalled that HAUSER AND SWEARINGEN found a pronounced minimum in the surface tension of egg albumin at the isoelectric point in the absence of neutral electrolytes. Evidently, the addition of electrolytes abolishes this minimum. CRUMPER AND ALEXANDER[7] in their work on spread films of β-globulin and of pepsin at a white oil–water interface report a small maximum of film pressure at the respective isoelectric points of these proteins at an ionic strength of 0.03.

TABLE V

EFFECT OF 0.025 M NaCl ON THE ADSORPTION OF BSA ON EMULSIONS OF *n*-OCTADECANE AT 30°

pH	*Absence of salt*		*0.025 M NaCl*	
	mg/M²BSA	*Stability emulsion*	*mg/M²BSA*	*Stability emulsion*
2.8	3.1	Unstable	4.9	Stable
5.0	7.0	Stable	6.2	Stable
9.5	2.9	Unstable	4.2	Stable

The pronounced influence of the ionic strength on the tensions of protein solutions at pH values far removed from the isoelectric points of the protein, may be due to a relatively low adsorption at such pH values in the absence of salt. The lower adsorption values may in turn be due to electrostatic repulsion between the protein molecules which would be expected to decrease with increasing ionic strength. The extent of adsorption of BSA on *n*-octadecane emulsions has been measured in the absence and presence of 0.025 M NaCl and at the indicated pH values (see Table V).

There is an indication from Table V that higher adsorption occurs near the isoelectric point with or without NaCl and also an increased adsorption and stability at pH 3 and 8 upon the addition of NaCl. Under all conditions, the amount of protein adsorbed is always more than enough to cover the interface completely.

Since the interfacial tensions between *n*-octadecane and BSA solutions have been measured at two different temperatures (30° and 40°) (see Table III), enthalpy and the entropy change per square centimeter of surface can be calculated by conventional means. In Fig. 5 is shown the entropy change expressed in ergs/degree/cm² as a function of the concentration of BSA. At lower concentrations of BSA there is an increase in randomness at the interface possibly associated with the surface denaturation of the BSA. At still higher concentrations of the protein, the entropy change for expansion of the surface becomes negative which is probably related to an ordering process in the condensed protein film both in respect to the protein and to the water.

The addition of octadecyl amine to the *n*-octadecane even in quite small concentrations has a profound effect on the interfacial tension between water and *n*-octadecane both with and without the addition of BSA (see Table IV). Evidently, octadecylamine is capillary active at the paraffin–water interface and would, therefore, accumulate at the interface. Considerable interaction between the adsorbed layer of octadecylamine situated on the paraffin side of the interface with the BSA adsorbed on the water side is to be expected and probably there has been complexing between the BSA and the amine with mutual penetration of the two adsorbed films. The interfacial-tension changes no doubt reflect a complex situation which is difficult to analyze. It should be added, however, that parallel studies on the adsorption of BSA on *n*-octadecane emulsions containing octadecylamine shows that there is no decrease in the amount of BSA adsorbed as the concentration of the octadecylamine is increased.

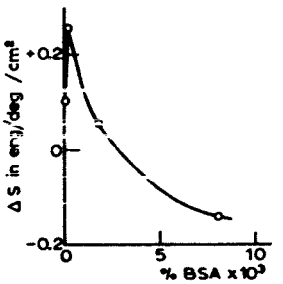

Fig. 5. Entropy change for the interface between *n*-octadecane and BSA solutions as a function of the BSA concentration at pH 5.9, and ionic strength 0.05 at 30°.

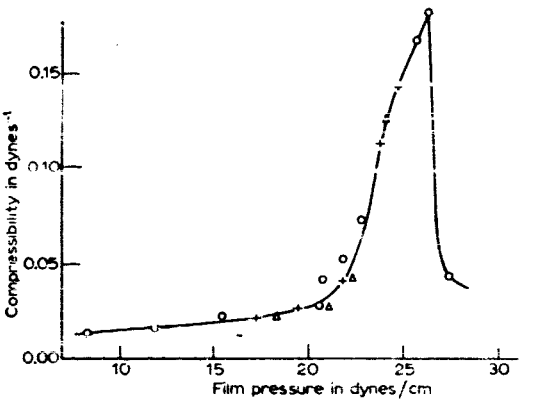

Fig. 6. Compressibilities in reciprocal dynes plotted against the corresponding film pressures for BSA films adsorbed at the solution–*n*-octadecane interface at pH 4.2, ionic strength 0.05 and 30°. ○, $2.7 \cdot 10^{-4}$% BSA; +, $9.0 \cdot 10^{-4}$% BSA (pH 5.90); △, $9.0 \cdot 10^{-4}$% BSA

The pressure–area relationships shown in Fig. 4 are of interest since a direct determination of such relationship for an adsorbed film of protein has never been attempted before. The curves show a plateau region at about 25 dynes/cm, representing the collapse of the film. It is difficult to compare such pressure–area curves with those obtained with a spread monolayer of protein, since the amount of the protein at the interface is not known. However, if one determines the adsorption of BSA on *n*-octadecane emulsions under the same conditions of pH and equilibrium BSA concentration as specified in the legend of Fig. 4, the area occupied by the adsorbed protein is always found to be less than 0.40 m²/mg, and in the higher film pressure region, lies between 0.20 and 0.25 m²/mg. These areas are much smaller than that occupied by a spread monolayer of protein at low pressures but are comparable to those of films spread at high initial pressures as in the experiments of LANGMUIR AND WAUGH[8], HAUROWITZ *et al.*[9], JOLY[10] and especially of AUGENSTINE *et al.*[11,12].

It is possible to calculate the coefficient of compressibility of the adsorbed interfacial films from the results shown in Fig. 4; the coefficient of compressibility has been set equal to $1/A \cdot dA/d\pi$ where A is the relative area of the interface and π is the film pressure in dynes/cm. Fig. 6 shows the plot of the compressibilities against

the film pressure. The similarity of the compressibility coefficients of the protein film for a given film pressure and irrespective of the concentration of the BSA solutions indicates the structure of the film to be a function of the film pressure only and not of the protein concentration.

An inspection of Fig. 6 shows that the compressibilities of the protein film at the higher protein concentrations are comparable with the compressibilities of the F – C films of trypsin noted by AUGENSTINE *et al.*[12]. The AUGENSTINE results also exhibit a considerable increase in the compressibility coefficients above a film pressure of about 22 dynes/cm. It seems likely that films of BSA adsorbed at a paraffin–water interface resemble closely the F – C films of AUGENSTINE obtained by spreading trypsin against relatively high film pressures.

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