Fundamental Study of Biochemical Behavior of Anionic Sulfonate- and Sulfate-Type Surfactants

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ABSTRACT

The interactions of α-olefin sulfonate with proteins were studied and a few environmental and hygienic phenomena investigated on the basis of these interactions. It was found that α-olefin sulfonate formed a complex with proteins mainly through ionic bonds similar to those formed by linear alkylate sulfonate and alkyl sulfate and that the α-olefin sulfonate-protein complex had much in common with these surfactant-protein complexes. The data on surfactant adsorption indicate that the effects of sulfonate-type surfactants are due to the formation of complexes with the protein of the skin, hog bristles, and human hair. Adsorption of surfactant occurred in the gills of fish; the adsorption increased with time. When protein was added, the surfactant solution was less biologically effective to fish. The data suggest that adsorption of the surfacntant disturbs the functions of fish gills. Increase in erythrocyte count, which always is found in oxygen-deficient conditions, occurred in fish killed by exposure to the surfactant.

INTRODUCTION

In many previous investigations on interaction between anionic surfactants and proteins, the formation of surfactant-protein complex has been reported (1-4). Anionic surfactants of the sulfonate- and sulfate-type, but mainly linear alkylate sulfonate (LAS) and alkyl sulfate (AS), complex with proteins primarily through ionic bonds (5,6). There are two or three types of complexes depending upon the conditions of interaction (7). Previous investigations have shown that complex formation changes the protein structures and inhibits enzymatic activity (4,7,8).

The present paper shows, by the use of gel filtration, electrophoresis, and equilibrium dialysis techniques, that α-olefin sulfonate (AOS) forms the protein complex mainly through ionic bonds similar to those formed by LAS or AS and that the AOS-protein complex has much in common with these surfactant-protein complexes. On the basis of a previously reported biochemical study by the author, the behavior of surfactant-protein complexes is involved in processes, such as foaming in rivers (9), removal of natural soil (10,11), sweet potato starch refining (12), and extraction of heavy metals from contaminated rice (13). This report also deals with a few other environmental and hygienic phenomena involving the behavior of the complexes.

EXPERIMENTAL PROCEDURES

Materials

AOS was prepared from α-olefin consisting of 15-18 carbon atoms by sulfonation with sulfur trioxide and hydrolysis with sodium hydroxide. The resulting mixture of 60% sodium alkenylsulfonate and 40% sodium oxyalkylsulfonate (99.2% of active ingredients) decomposed at over 300 C. In this study, aqueous solutions were used. AOS-C^{14} and LAS^{35} were obtained from Daiichi Pure Chemicals Co., Tokyo, Japan. Bovine serum albumin (BSA) and human serum albumin (HSA) were obtained from Merck & Co., Darmstadt, Germany. Rice protein, extracted from polished rice with a 0.1% sodium hydroxide solution, was obtained as a precipitate by adjusting the pH of the solution.

Experiments on Surfactant-Protein Complex

Gel filtration: A solution containing 5.2 x 10^{-4} mole/liter AOS and 4.6 x 10^{-5} mole/liter BSA was put on a Sephadex G-50 chromatographic column (2 x 22 cm) and eluted with a Sörensen buffer solution, pH 7.4, M/15, μ = 0.17, and 5 ml fractions were obtained. The quantity of BSA in each fraction was determined by measuring optical density at 280 mμ and the amount of AOS determined by methylene blue colorimetric analysis.

Electrophoresis: The complex formation of AOS, at its high concentration (2.96 x 10^{-2} mol/liter) with HSA, was studied paper electrophoretically. AOS-HSA complexes were developed in a solution containing 1% of AOS and 2% of HSA within the pH range of 7.0-7.5 and kept at room

TABLE I

Adsorption of Linear Alkylate Sulfonate (LAS) on the Human Skin by Various Treatments

Treatment (dipping)		Amounts of adsorbed LAS
Pretreatment[a]	Post-treatment[b]	(μg/cm²)
With LAS solution (pH 5.0)	None	19.54
With LAS (pH 5.0)	With water (pH 7.0)	18.87
With LAS (pH 5.0)	With alkali solution (pH 11.0)	9.64
With LAS (pH 5.0	With toilet bar soap solution	8.67
With LAS (pH 5.0)	With glycerin and potash solution (pH 12.8)	6.29

[a]Dipped for 15 min.

[b]Dipped twice for each 1 min.

TABLE II

Adsorption of Linear Alkylate Sulfonate (LAS) on Hog Bristles and Human Hair

Samples	Treatment time with LAS[a] (hr)	Amount of LAS adsorption, μg/cm²	
		Post-treatment-1[b]	Post-treatment-2[c]
Hog bristles	48	3.28	2.57
	96	3.98	3.54
Human hair	48	15.70	8.78
	96	17.12	12.75

[a]Stirring in a Terg-O-Tometer in a 0.8% LAS^{35} solution.

[b]Rinsed for 1 min in water after LAS treatment.

[c]Rinsed for 1 min in water after LAS treatment and further treated with a hair rinse for 5 min and then rinsed for 1 min in water.

TABLE III

Effect of Linear Alkylate Sulfonate (LAS) upon Water Retention of Human Hair

Samples	Water content (%)[a]	Recovery water content (%)[b]
Untreated	12.0	92.10
Treated with LAS[c]	13.89	92.36
Treated with water[d]	13.65	93.50

[a]Conditioned at 20-25 C, at 65% relative humidity for 24 hr and then dried for 4 hr at 105 C. Wt loss measured as water content.

[b]Conditioned again at 20-25 C, at 65% relative humidity for 24 hr after drying above mentioned. Wt gain measured as water recovery.

[c]Dipped in a 10% LAS solution for 24 hr at 40 C and then rinsed in water to remove LAS.

[d]Dipped in water for 24 hr at 40 C.

TABLE IV

Effect of Linear Alkylate Sulfoante (LAS) upon Strength and Elongation of Human Hair

Samples	Tensile[a] strength (Kg/mm²)	Elongation[a] (%)
Untreated	49.78 ± 4.7	43.3 ± 1.7
Treated with LAS[b]	48.50 ± 3.9	39.7 ± 3.0
Treated with water[b]	47.60 ± 3.2	41.3 ± 2.0
		(n = 40)

[a]Measured with Tensilon meter under the following conditions: chuck distance = 30 mm and cross head speed = 50 mm/min.

[b]Treated in the same manner as mentioned in Table III.

temperature, the wt ratio of HSA-AOS being 1:1/2. This mixed solution was dialyzed in a stream of tap water for ca. 1 hr and then in the distilled water in a cold room (5 C) overnight. The resulting precipitate, surplus AOS, was removed by centrifuging (1085 G, 15 min). Complexes with other wt ratios were prepared in a similar manner and subjected to electrophoresis in a phosphate buffer solution (pH 6.8, $\mu = 0.13$) for 1 hr at 0.5 mA/cm using a cellulose acetate membrane and dyed with a 0.5% Ponceau 3R solution.

Equilibrium dialysis: An AOS solution of 3.62×10^{-5} mole/liter was added to a rice protein solution having a nitrogen content of 0.52 mg/ml and dialyzed for 7 days against buffer solutions of various pH levels. AOS in the solution outside the bag was determined by methylene blue colorimetric analysis. Buffer solutions included were as follows: M/5 Clark-Lubs pH 1.0, 3.0, 5.0, 6.0, 7.0, 8.0, 9.0, and M/10 Kolthoff pH 11.0.

Experiments on Human Skin

Fingers were dipped for 15 min in a 0.04% LAS solution of pH 5.0, having a specific radioactivity of 0.16 μCi/ml, then rinsed twice for each 1 min in each of the following solutions: water, glycerin-potash solution (Baelz's solution), 1% soap solution of pH 10.5, and an alkali solution of pH 11.0. LAS remaining on the fingers was extracted twice for 5 min in 100 ml 70% ethanol solution of pH 10.0. The ethanol then was removed by distillation and 20 ml scintillator (Insta-Gel, Packard Instrument Co., Downers Grove, Ill.) added to the residue. LAS^{35} was determined by means of a liquid scintillation counter (Packard model 3380 Tri-Carb).

Experiments on Hair

Hog bristles and human hair were treated with 0.8% LAS^{35} solution by stirring in a Terg-O-Tometer for 48 and 96 hr, rinsed for 1 min in water to remove loosely bound surfactant, and then further treated with a hair rinse. The LAS^{35} remaining on the treated hog bristles and human hair was extracted with ethanol at 50 C and was measured by a liquid scintillation counter. The treated samples also were submitted to microautoradiography.

Human hair treated with 10% LAS for 24 hr was conditioned at 20-25 C (65% relative humidity) for 24 hr, then dried for 4 hr at 105 C, and analyzed for retention of water. Tensile strength and elongation tests also were performed under the following conditions: chuck distance, 30 mm; and cross head speed, 50 mm/min.

Experiments on Fish

Ten goldfish (*Carassium auratus*) were kept in 10 liter 10 ppm AOS solution with no aeration for 0.5, 1, and 3 hr. After dissection, the gills and alimentary canals were extracted in ethanol to determine the level of AOS adsorption.

Ten goldfish were kept in 10 liter 20 ppm AOS-C^{14} solution (total 1.27 mCi) for 30 min or 3 hr, then frozen, and sliced by a microtome. The prepared slices were examined by autoradiography.

Three carp (*Cyprinus carpio*) were exposed to 10 liter 20 ppm and 80 ppm LAS solution. Three goldfish also were treated in the same manner. As soon as they died, their blood was collected with an injector and erythrocyte counts made using a Coulter counter.

Japanese killifish (*Oryzias latipes*) were placed in 5 ppm AOS containing 2100 ppm egg albumin and 10 ppm AOS containing 2100 ppm or 4200 ppm egg albumin. The percentage of their survival was checked at 0.5, 1, 2, 3, 6, 12, 24, and 48 hr. Dead fish were removed as death occurred during the test period.

RESULTS

Formation of Surfactant-Protein Complexes

Gel filtration of AOS is represented by its own elution in the no. 20 and adjacent fractions and also in fractions no. 5 and 6. Since the no. 5 and 6 areas agree with that of the BSA elution fractions, it may be assumed that AOS in these fractions is complexed with BSA. The BSA elution pattern

was not affected. In the bonding of AOS to rice protein, the formation of the complex is influenced by pH of the medium. The percentage of bonded AOS falls as the pH rises from 1-7; at pH 9 and beyond, the curve levels off. Since over 80% of the AOS is bonded to the protein even beyond pH 9, it is assumed that hydrophobic bonds, as well as ionic bonds, are involved.

In the electrophoretic mobility of the AOS-HSA complexes, as the wt ratio of AOS/HSA was changed from 1/2 to 3, the mobility increased from 7.2 to 8.9 x 10^{-5} cm^2/V sec. The ratio of 4 or above showed the mobility of 9.4 x 10^{-5}. Both halves of a cut spot showed color reactions for HSA and AOS and a specific degree of mobility different from that of HSA alone, i.e. 6.7 x 10^{-5}, indicating qualitatively the formation of an AOS-HSA complex.

The numbers of anionic surfactants, AOS, LAS, AS, and alkyl ethoxy sulfate (AES), bonded to protein in the interaction of surfactants and protein were measured by use of equilibrium dialysis. In each case, the equilibrium constant k was determined by Scatchard's formula (14): $\gamma/A = kn - k\gamma$ (where γ is the number of bonds, and A, concentration of the surfactant).

The fact that the bond free energies of the 4 anionic surfactants calculated from these equilibrium constants were similar to one another. Ca. 7600-8800 cal/mol, suggests that they combine with protein in much the same manner.

Effects upon Human Skin

It was found that dishwashing detergent solutions have a pH of ca. 5 and that detergent solutions for washing clothes have pH values of 9.5-9.8. Some professional users of surfactants have learned from experience that they can prevent and remove abnormal skin sensations after using surfactants by washing their hands with toilet soap. Therefore, it was assumed that sulfonate- or sulfate-type surfactants actively combine with protein to form a complex at ca. pH 5 and that the surfactant remaining on the user's hands results in the skin sensations. Evidence for this is supported by Table I which shows the adsorption of LAS^{35} on the skin. The large amount of LAS^{35} remaining on the skin cannot be removed easily by simply washing with water, but over 50% of it can be removed by treating the hands with a high alkali soap solution or Baelz's solution.

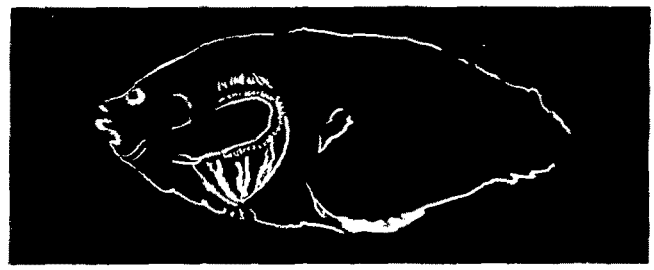

FIG. 1. Macroautoradiographic view of goldfish kept in a α-olefin sulfonate solution for 3 hr.

Effects upon Hog Bristles and Human Hair

Some sulfonate- and sulfate-type surfactants are used widely for washing hair. Although there is anxiety that they might cause the hair to lose moisture and tension, they present no problem in ordinary use. As has been reported, treatment under extreme conditions, e.g. high surfactant concentration, high temperature, long treatment time, or special hair dying is liable to affect the hair (15), so the effect of surfactants upon hog bristles, as a simulated model, and upon human hair is studied under extreme conditions.

The results shown in Table II indicate that the longer treatment with LAS resulted in more adsorption of surfactant and that, in the case of human hair, very little was removed by treatment with a hair rinse.

Tables III and IV show that the moisture retention, tensile strength, and elongation properties of treated and untreated human hair are ca. the same, even under the extreme conditions.

Effects upon Fish

Figure 1 is a autoradiograph of the fish kept 3 hr, and Table V shows that the adsorption of AOS occurs in the gills of goldfish. AOS adsorption in the gills increases with time, but no adsorption was noted in the alimentary canal.

TABLE V

Adsorption of α-Olefin Sulfoante (AOS) on Gills and Alimentary Canals of Goldfish[a]

Organs	Residence time of fish in a 10 ppm AOS solution (hr)	Amount of adsorbed AOS (μg/g organ)
Gills	0.5	0.3
	1	2.5
	3	48.3
Alimentary canals	0.5	0.0
	1	0.0
	3	0.0

[a]This experiment was carried out by use of AOS-C^{14}.

TABLE VI

Change of Erythrocyte Counts of Carp and Goldfish upon Exposure to Linear Alkylate Sulfonate (LAS)

Fish	Exposure solution	Erythrocyte counts[a] (x $10^4/mm^3$)	Time up to death[b]
Carp	Water	78	No deaths (>48 hr)
	80 ppm LAS solution	127	30 min
	Water added dry ice	117	1 min
Goldfish	Water	133	No deaths (>48 hr)
	20 ppm LAS solution	173	90 min

[a]Mean value obtained from three fish in each case.

[b]All of the exposed fish were dead after these time intervals.

TABLE VII

Fish Toxicity of α-Olefin Sulfonate (AOS) Alone and of AOS with Protein Added[a]

Test solutions	L_TO (hr)[b]	L_T100 (hr)[c]
AOS (5 ppm)	0.5	2
AOS (5 ppm) plus egg albumin (2100 ppm)	>48	——
AOS (10 ppm)	0.5	1
AOS (10 ppm) plus egg albumin (2100 ppm)	2	3
AOS (10 ppm) plus egg albumin (4200 ppm)	2	6

[a]Ten Japanese killifish were used for each experiment.
[b]L_TO: 100% survival (time).
[c]L_T100: 100% dead (time).

On the basis of these data, it is assumed that the biological effects of sulfonate-type surfactants upon fish are related to adsorption in the gills, i.e. to surfactant bonding with the gill protein. Previously reported data show that the erythrocyte count of fish deficient in oxygen increases very rapidly (16). Presumably, surfactant adsorption causes the gills to become less functional, thus resulting in an oxygen deficiency which causes the fish to die. Table VI shows the erythrocyte count for fish treated with surfactant and dry ice.

A general increase in erythrocyte count is noted in fish killed by exposure to LAS—a condition paralleling ordinary cases of oxygen deficiency. Presumably, the biological effects of sulfonate-type surfactants are related to their bonding to the gill protein.

Table VII shows that the addition of egg albumin to the surfactant solution minimizes the biological effect of the surfactant. As pointed out previously, sufonate-type surfactants cause an oxygen deficiency in fish by adsorption in the gills. Presumably the surfactant-protein complex interferes with the oxygen supply mechanism.

DISCUSSION

Sulfonate-type surfactants, in dishwashing solutions having a pH value of ca. 5, combine with skin protein to form a complex which remains on the skin. It might be thought that the care treatment of some professional users of surfactants, i.e. rinsing with soap after their work, is based upon removing surfactants adsorbed on the skin by an alkali solution.

When hog bristles and human hair were treated with a sulfonate-type surfactant solution, the amount of surfactant adsorbed increased with the increase in treatment time. The adsorption appears to be due partly to the combination of the surfactant with hair protein. Under the extreme conditions, the tensile strength and elongation did not show any significant difference although they tended to decrease somewhat. Complexes formed by the interaction of a sulfonate-type surfactant and human hair protein seemed to have no effect upon the physical properties of the hair.

The biological effect of sulfonate-type surfactants to fish is related to the adsorption of surfactant in the gills where a surfactant-protein complex is formed. This complex apparently interferes with the respiratory function of the gills, thus causing an oxygen deficiency as evidenced by a parallel increase in erythrocyte count. However, when a foreign protein (egg albumin) was added to a surfactant solution containing fish, the effect was lowered, because the surfactant apparently was complexed with the previously added protein. Therefore, it may be concluded that the biological effect of sulfonate-type surfactants is due to the formation of surfactant-fish protein complexes.

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