Surface Active Betaines As Protective Agents against Denaturation of an Enzyme by Alkyl Sulfate Detergents

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ABSTRACT

A homologous series of higher alkyl sulfate surfactants inactivate β-fructofuranosidase (invertase) at levels coinciding with their critical micelle concentrations. It was possible to renature the enzyme by passing it through an anion exchange column. This inactivation was prevented by surface active betaines present at equimolar or higher concentrations than those of the anionics. Effective surfactant betaines include those with carboxylate, sulfonate, or phosphate radicals in their zwitterions. Betaines lacking surface active properties did not prevent denaturation indicating that the effects are due to comicellization. Studies with enzymes may point to appropriate anionic/zwitterionic surfactant ratios in solubilization procedures or detergent applications where biological properties must be preserved and anionic surfactants are required as components.

INTRODUCTION

Sodium n-dodecyl sulfate (C_{12}sulfate) has been employed widely in protein extractions from biological membranes (1-6). This detergent frees membranes of lipoprotein subunits (2,3), but the proteins are generally denatured (4-7). Invertase is denatured by C_{12}sulfate under acidic conditions if not protected by substrate (7,8) and is not renatured by subsequent increase in pH.

The effects of n-octyl sulfate (C_8sulfate) and n-decyl sulfate (C_{10}sulfate), on this enzyme have not been reported previously.

Zwitterionic surfactants of the betaine type generally preserve enzymatic properties (9-11) and are superior to nonionics as membrane solubilizers (9). This latter class of surfactants are also considered "mild" to proteins.

In the work reported here, surfacant effects on yeast invertase were evaluated with n-alkyl sulfates, 3-(n-alkyldimethylammonio)-1-propanesulfonates(sulfobetaines), (n-alkyldimethylammonio)-acetates, α-(trimethylammonio)-dodecanoate (carboxyalkylbetaines) and lysolecithin (a phosphobetaine).

Sulfobetaines retain their zwitterionic character regardless of pH. Unique among amphoterics, they are not retained when passed through anion- or cation-exchange resins or mixed-bed resins (12,13). However, the carboxyalkylbetaines can acquire cationic properties when protonated at low pH. If ionic interaction between invertase and surfactant is a required step in its denaturation (8), at least the sulfobetaines could be expected to prove inert.

EXPERIMENTAL PROCEDURES

Preparation and Purification of Surfactants

Sulfobetaines, 3-(alkyldimethylammonio)-1-propanesulfonate (Table I) and sodium alkyl sulfates (Table II) were prepared and purified as described previously (12). Carboxyalkylbetaine,

α-(Trimethylammonio)-dodecanoate $H_3C\text{-}(CH_2)_9\text{-}CH(N^{\oplus}(CH_3)_3)\text{-}C(=O)\text{-}O^{\ominus}$

was prepared by a published method (14) and purified

TABLE I

List of Employed 3-(Alkyldimethylammonio)-1-Propanesulfonate Surfactants

n-Alkyl group	Molecular weight Theory	Molecular weight N-basis (Kjeldahl)	-Sulfobetaines
Octyl-	279.4	268.1	C_8-
Decyl-	307.4	309.8	C_{10}-
Dodecyl-	335.6	337.5	C_{12}-
Tetradecyl-	363.6	370.2	C_{14}-

TABLE II

List of Employed Sodium Alkyl Sulfates

n-Alkyl group	Molecular weight theory	Methylene blue activity, %	-Sulfate
Octyl-	232.3	98.7	C_8-
Decyl-	260.2	98.9	C_{10}-
Dodecyl-	288.4	99.9	C_{12}-

TABLE III

List of Employed Carboxyalkylbetaines

Compound	Percent nitrogen - Theory	Percent nitrogen - Kjeldahl	Equiv. weight, by titration[a]	-Carboxylmethylbetaine
(Decyldimethylammonio)-acetate, monohydrate	5.35	5.31	263	C_{10}-
(Tetradecyldimethylammonio)-acetate, monohydrate	4.42	4.50	310	C_{14}-
α-(Trimethylammonio)-dodecanoate	5.44	5.32	262	

[a]In glacial acetic acid with 0.1 N perchloric acid, methyl violet indicator.

through repeated extraction of a 20% aqueous surfactant solution with ca. 50% (v/v) of heptane until the extractant no longer deposited residue on evaporation. The aqueous layer was filtered through a Millipore filter (0.45 μ) and dried at 60 C in vacuo. Solids were then redissolved in boiling anhydrous ethanol, filtered and dried as before and analyzed (Table III).

(Alkyldimethylammonio)-acetate, monohydrate surfactants, $H_3C\text{-}(CH_2)_n\overset{CH_3}{\overset{|}{\overset{\oplus}{N}}}\text{-}CH_2\text{-}\overset{O}{\overset{\|}{C}}\text{-}O^{\ominus}\cdot H_2O$, were synthesized as described in U.S. patent 2,082,275 (15) with fractionally distilled n-decyldimethylamine or n-tetradecyldimethylamine. The reaction products were then diluted to 10% solids with 50% aqueous ethanol (v/v), purified as described above and analyzed (Table III).

Lysolecithin, from phospholipase A-treated egg lecithin, type I, was obtained from Sigma Chemical Company.

Nonsurfactant Betaines

Betaine hydrate was purchased from Eastman Organic Chemicals. 3-(Trimethylammonio)-1-propanesulfonate, $CH_3\overset{CH_3}{\overset{|}{\overset{\oplus}{N}}}\text{-}(CH_2)_3\text{-}SO_2O^{\ominus}$ was prepared in a reactor as described in U.S. patent 3,280,179 (16): 61.2 g (0.5 moles) propanesultone were added dropwise to 146.6 g (0.625 moles) of 25% aqueous trimethylamine at a rate to control the exotherm below 40 C (caution: propanesultone has hazardous properties). After stirring the solution for 2 hr, the temperature was gradually raised to 106 C and the solution refluxed for 4 hr. Excess amine was removed by sparging with nitrogen at the reflux temperature. On cooling, the pH of the reaction mass dropped to below 2. For purification the solution was passed through a mixed-bed ion exchange resin (Amberlite MB 1, Rohm and Haas Company). The eluate was filtered and dried at 60 C in vacuo. A white, hard crystalline solid remained and was recrystallized twice from dry acetone. Nitrogen content by micro-Kjeldahl was 7.9%, compared with the theoretical value of 7.74. The product is completely soluble in water but insoluble in chloroform. An infrared spectrum was obtained through a film cast from aqueous solution on a AgCl window. Sultone absorption bands (10.3 μ and 11.2 μ) were absent.

Determination of Critical Micelle Concentrations (CMC)

CMC of anionic surfactants was determined by dye solubilization, using pinacyanol chloride (17). A solution of 2.5 mg enzyme per ml buffer containing approximately double the CMC of surfactant under test and 5 x 10^{-2} mM pinacyanol chloride was stirred while being diluted with the same, but surfactant-free, solution dispensed from a burette. The surfactant-containing portion was thus diluted gradually to the CMC which resulted in a change of color from an intense blue (at or above the CMC) to a pale violet (below the CMC). This method also proved useful in determining micellar concentrations of the surfactant betaines used in this work. Thus, the CMC of C_{10} carboxymethylbetaine gave the typical color change at 19-20 mM in distilled water at 25 C, comparing favorably with reported values (18,19). Similarly, C_{12}sulfobetaine showed a CMC rnage of 4.5 to 4.7 mM which is in line with the value found through light scattering by a previous investigator (20). Pinacyanol chloride was obtained from Eastman Organic Chemicals.

Ion Exchange Resin for Renaturing of Enzyme

Invertase denatured by alkyl sufate was renatured with Dowex AG-1X2 anion exchange resin. The resin (0.5 g dry basis), acetate form, in disposable columns (10 mm x 40 mm) equilibrated with distilled water was obtained from Bio-Rad Laboratories. Water was drained prior to use and 2 ml of the denatured invertase passed through the column over a period of 10-15 min. Concentration of the recovered invertase for subsequent sucrose hydrolysis was adjusted based on the optical density at 280 nm of the renatured invertase solution.

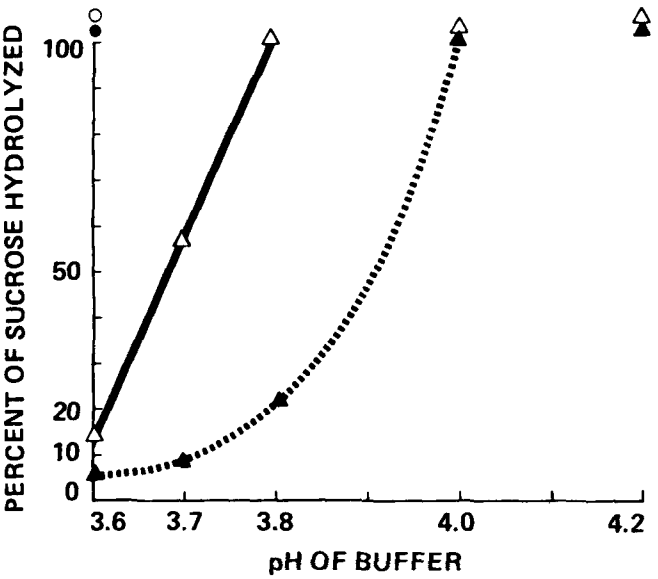

FIG. 1. Effects of pH and temperatures on the inactivation of invertase (5 mg/ml) following incubation of one hour in acetate buffer, containing 2.0 mM sodium dodecyl sulfate. Values given are percent sucrose hydrolyzed by treated invertase compared with enzyme incubated in buffer only (control). △: Incubated in detergent at 24 C; ▲: Incubated in detergent at 37 C; ○: Incubated in buffer only at 24 C; ●: Incubated in buffer only at 37 C.

Enzymes

Invertase from bakers yeast (172 I.U./mg at 30 C, pH 4.5) was purchased from Calbiochem-Behring Corporation. Phosphoglucose isomerase (1992 I.U./ml at 30 C, 4 mg protein/ml) from yeast was obtained from Calbiochem-Behring Corporation as a crystalline suspension in 3.2 M ammonium sulfate.

Incubation of Invertase with Surfactants

Invertase was dissolved in buffer (0.1 M acetate, pH 3.80) containing the surfactant, and the pH was adjusted as required. Aliquots of these solutions were incubated for 1 hr at 24 C and 37 C. All incubations, subsequent hydrolyses and assays were replicated two or three times. The results are averages of these.

Sucrose Hydrolysis

Each ml of hydrolysis solution contained 0.323 mg of invertase (as a solution from the incubation mixture) and 5.21 mg sucrose in 0.1 M acetate buffer (pH 3.80 ± 0.02). Hydrolysis was stopped by mixing 100 µl of the hydrolysis solution with 100 µl of 16 mM C_{12}sulfate solution in 0.1 M acetate buffer (pH 3.80). Glucose levels remained constant when observed over a period of 3 hr.

Assay

Hydrolysis of sucrose was determined spectrophotometrically by measuring the level of glucose and fructose (21).

Circular Dichroism Spectra

These were obtained with a Durrum-Jasco ORD/UV-5 CD spectrophotometer and recorder. The path length of the cell was 0.5 cm.

RESULTS

Effect of Sodium Alkyl Sulfates

Homologous alkyl sulfates, selected to have Krafft points (22) below the lowest incubation temperature (24 C), were evaluated for their effect on yeast invertase. Preliminarily, this glycoprotein (5 mg/ml) was incubated at 24 C and 37 C for 1 hr in buffer solutions containing 2 mM C_{12}sulfate over a range of pH 4.2 to pH 3.6. Clear differences regarding the inactivation of this enzyme over a narrow pH range and at the two temperature levels became evident. When invertase was incubated at pH 3.80 and 24 C, it hydrolyzed sucrose quantitatively, but hydrolysis dropped to ca. 22% when the incubation temperature was raised to 37 C (Fig. 1). At pH 4.0 or above, the incubated enzyme hydrolyzed sucrose quantitatively at both 24 C and 37 C. However, activity was almost completely eliminated at either temperature when the pH was reduced to 3.6 (Fig. 1). Hydrolysis was complete in all surfactant-free controls (Fig. 1). All subsequent hydrolyses were conducted at pH 3.80 ± 0.02.

Inactivation of invertase by C_{12}sulfate was compared with the effect of lower sodium alkyl sulfates. Each surfactant was tested at concentrations ranging from 25% to 200% of its CMC with 2.5 mg invertase per ml of incubation solution. It was interesting to note that denaturation coincided in each case with the CMC or its threshold when incubations were carried out at 24 C (Fig. 2). In two cases it occurred at the next lower concentration when incubations were at the higher temperature (Fig. 2). The pinacyanol chloride method (17) was used to establish the CMC of incubation mixtures containing buffer, enzyme and surfactant. These were moderately lower than values reported for distilled water solutions (23) and showed doubling of CMC for each reduction of one methylene group of the alkyl chain (24).

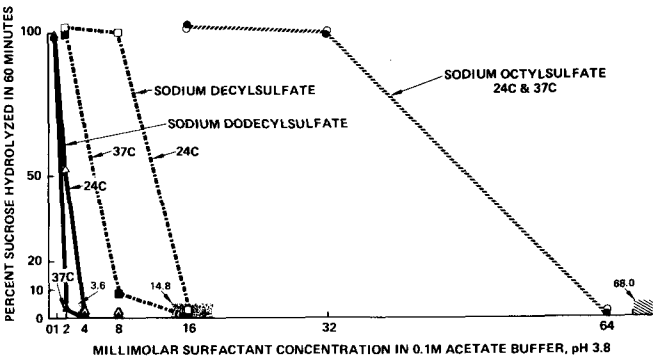

FIG. 2. Hydrolysis of sucrose by invertase, following incubation of 2.5 mg enzyme/ml for one hour at 24 C and 37 C in alkyl sulfate detergents below and within their CMC. Loss of activity coincides with CMC of the incubation mixture (shaded area) in each of the three surfactants examined at incubation temperatures of 24 C and occurs at slightly lower concentration in two cases at 37 C. Filled symbols represent the higher temperature.

Effect of Surface Active Betaines on Invertase

The enzyme was incubated with surface active betaines, selected to have sulfonate(sulfobetaines), carboxylate (carboxyalkylbetaines) or phosphate (lysolecithin) radicals. Carboxyalkylbetaines were prepared to have the quaternary ammonium group as part of the molecular backbone, as in (dodecyldimethylammonio)-acetate, or as a pendant group, as in α-(trimethylammonio)-dodecanoate (Table III). Surfactant levels below and above the CMC were selected. None of the surfactant betaines denatured invertase at any of the concentrations tested and the hydrolysis of sucrose was quantitative in each case (Table IV).

Rate of sucrose hydrolysis was not reduced by incubation of invertase (2.5 mg/ml) with 8 mM C_{14}sulfobetaine nor was it adversely affected when the same amount of enzyme was exposed to 2 mM C_{10}sulfate (Fig. 3). The latter represents a level which is substantially below the CMC.

Effect of Mixed Micelles of Anionic/Zwitterionic Surfactants on Invertase

Surface active betaines, regardless of type employed, prevented denaturation of invertase by 8 mM or 16 mM C_{12}sulfate when the zwitterionics were present simultaneously in the incubation mixture at equimolar or higher concentration than the anionic surfactant (Fig. 4).

TABLE IV

Surface Active Betaine Incubation Solutions Which Do Not Denature Invertase

Betaine surfactant	Concentration tested, mM
C_{10}sulfobetaine	32, **64**[a]
C_{12}sulfobetaine	**1, 8, 32, 64**
C_{14}sulfobetaine	**1, 8, 32, 64**
α-(Trimethylammonio)-dodecanoate	**1, 8, 32**
C_{10}carboxymethylbetaine	**8, 32**
C_{14}carboxymethylbetaine	**0.1, 8, 32**
Lysolecithin (Egg white lecithin treated with lysine A)	**0.05, 8**

[a]Bold letters indicate micellar concentrations as established by the pinacyanol chloride method.

Similarly, the effect of 32 mM C_{10}sulfate or 64 mM C_8sulfate was reversed by surfactant betaines at the same molarity. Thus, invertase (10 mg enzyme/ml) incubated at 24 C with 8 mM C_{12}sulfate and zero to 16 mM C_{14}sulfobetaine hydrolyzed sucrose quantitatively in the presence of equimolar amounts of the two surfactants or with molar excess of the sulfobetaine, but not if the concentration of the anionic exceeded that of the sulfobetaine (Fig. 4). An excess of the zwitterionic was required when incubation was carried out at 37 C (Fig. 4).

A nearly identical profile developed when the sulfobetaine was replaced by C_{10}carboxymethylbetaine in the incubation solution. Here too, an equimolar quantity of the zwitterionic surfactant prevented denaturation by 8 mM C_{12}sulfate at 24 C, but a moderate excess of the carboxymethylbetaine was required to protect invertase against loss of enzymatic property when incubated at 37 C (Fig. 5). α-(Trimethylammonio)-dodecanoate, a betaine with pendant ammonium radical, confirmed the pattern of other surfactant betaines. An equivalent molar weight of lysolecithin preserved invertase activity at both 24 C and 37 C incubation temperatures. This phosphobetaine thus appears to be somewhat more efficient in this respect.

Effect of Betaines without Surface Active Properties

Betaine hydrate, (trimethylammonio)-acetate, monohydrate, or its sulfobetaine equivalent, 3-(trimethylammonio)-1-propanesulfonate, both lacking a lipophilic radical and therefore amphiphatic properties, were not able to block the denaturing effect of alkyl sulfates on invertase. This holds true even when the nonsurface active betaine was present in 100% excess.

Renaturation of Alkyl Sulfate Denatured Invertase

Invertase denatured with C_{12}sulfate could not be renatured by subsequent addition of surfactant betaines, even when the latter were present at 100% excess. Both surfactants must be present in the incubation mixture prior to addition of the enzyme for the protection effect of the surface active betaines to occur. Alkyl sulfate-denatured invertase could, however, be renatured by modification of a published method (25). Invertase (10 mg/ml) was first denatured by incubation with 8 mM C_{12}sulfate for 1 hr at 24 C and 37 C, respectively. Two ml of the enzyme solutions, which had lost all hydrolytic activity, were passed through columns containing Dowex AG 1-X2 anion exchange resin in the acetate form. The resin was previously equilibrated with water only. The eluates were of pH 6.4 to 6.6. To compensate for dilution of the invertase during chromatography, the amount of recovered enzyme solution employed in the sucrose hydrolysis step was adjusted based on absorptivity at 280 nm of the renatured invertase. Under these conditions, recovery of glucose and fructose from sucrose was quantitative, regardless of the temperature employed in denaturation.

Circular Dichroism Patterns

Circular dichroism patterns were obtained with 8 mM C_{12}sulfate and 8 mM C_{14}sulfobetaine-treated invertase. Incubation of the enzyme (1.0 mg/ml) was at 24 C in 2 x 10^{-2}M acetate buffer, pH 3.80. Treatment with C_{12}sulfate gave rise to a substantial increase in negative ellipticity, centered at ca. 207 nm compared to surfactant-free control. Invertase when incubated in buffer only shows a distinct shoulder at 234 nm which is not seen in the broader, more intense band of the alkyl sulfate-treated enzyme (Fig. 6). There was no discernible difference in the CD spectra of the sulfobetaine-treated enzyme and the control.

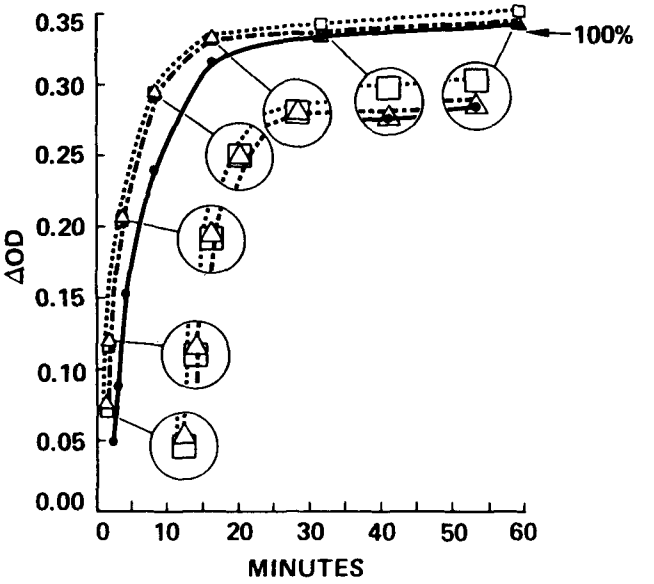

FIG. 3. Rate of sucrose hydrolysis (as ΔO.D., NADPH) by invertase, following incubation of 2.5 mg enzyme/ml for one hour at 24 C and pH 3.80 with two surfactants of type (sulfobetaine) or concentration (alkyl sulfate) which do not inactivate the enzyme. This is compared with surfactant-free control. △: Sodium decyl sulfate, 2 mM; □: 3-(Tetradecyldimethylammonio)-1-propanesulfonate, 8 mM; ●: Control, buffer only.

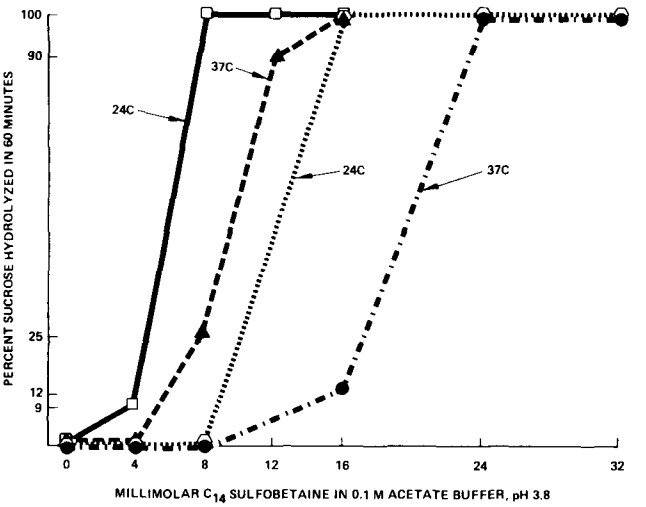

FIG. 4. Hydrolysis of sucrose by invertase following incubation of 10 mg enzyme per ml buffer, containing: □ ▬▬: 8 mM sodium dodecyl sulfate, at 24 C; ▲ ■ ■ ■: 8 mM sodium dodecyl sulfate, at 37 C; ○ ıılıılı: 16 mM sodium dodecyl sulfate, at 24 C; ● ı■ı■: 16 mM sodium dodecyl sulfate, at 37 C; and zero to 32 mM 3-(tetradecyldimethylammonio)-1-propanesulfonate (C_{14}sulfobetaine).

DISCUSSION

Invertase is denatured by 4 mM C_{12}sulfate at the low surfactant: enzyme weight ratio of 0.46:1 when the pH is below the enzyme's isoelectric range, reported to be 4.40-3.96 (26). However, four times this molar ratio was required to denature invertase with C_{10}sulfate and 16-fold such molar ratio eliminated activity with C_8sulfate. Thus, the concentration required to inactivate invertase is doubled for each decrease by one methylene unit of the alkyl chain. This is in line with micelle formation by homologous members of anionic surfactants (27,28). Indeed, the CMC for the buffer: enzyme incubation mixture was found to be 3.6 mM for C_{12}sulfate and 14.8 mM

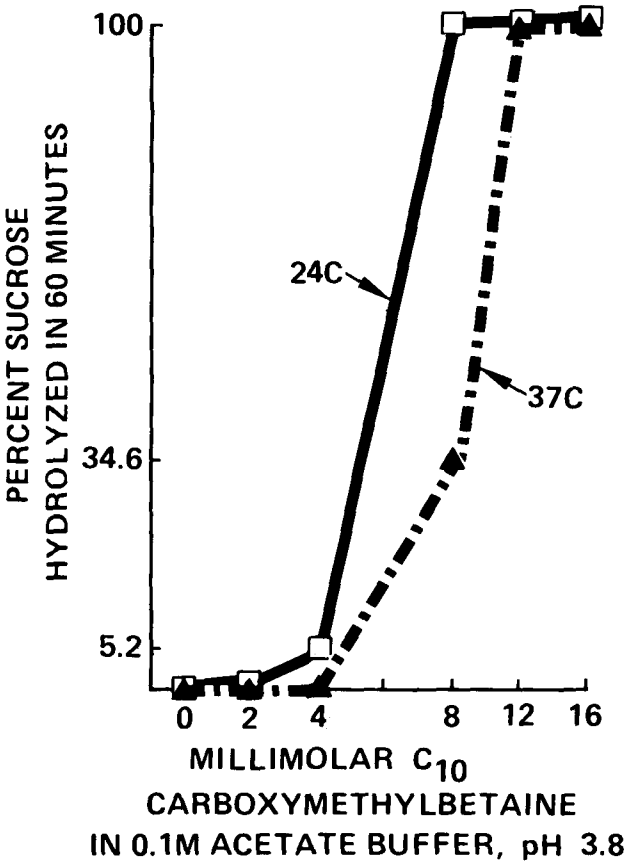

FIG. 5. Hydrolysis of sucrose by invertase following incubation of 10 mg enzyme per ml buffer, containing 8 mM sodium dodecyl sulfate and zero to 16 mM (decyldimethylammonio)-acetate (C_{10}-carboxymethylbetaine) at 24 C and 37 C, pH 3.8. □ ▬▬: C_{10}-carboxymethylbetaine, 24 C; ▲ ▮▬▮▬▮: C_{10}carboxymethylbetaine, 37 C.

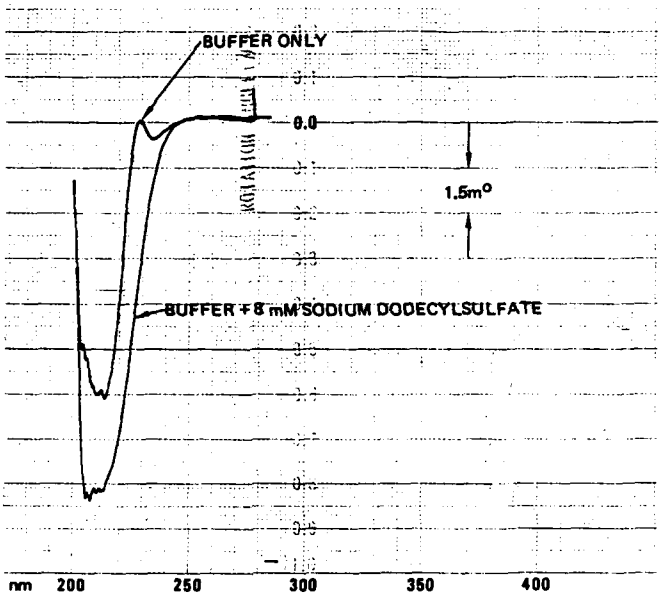

FIG. 6. The effect of 8 mM sodium dodecylsulfate on the circular dichroism spectrum of invertase (1.0 mg/ml) in 0.02 M acetate buffer, pH 3.8, following 1 hr incubation at 24 C. Results are compared with surfactant-free enzyme in buffer solution. Optical path length, 0.5 cm.

for C_{10}sulfate. The concentrations required to denature invertase with C_8sulfate and its CMC in the incubation mixture was only slightly higher than that predicted by such calculation. Therefore; it is clear that loss of enzymatic activity coincided with micellar concentrations of these anionics (Fig. 2).

The lack of effect by surface active betaines on invertase, at the broad range of concentrations and structural types demonstrated, is in contrast with the potent denaturing effect of alkyl sulfate surfactants. This is also reflected by the increased negative ellipticity, centered at 207 nm (Fig. 6), of circular dischroism spectra of the enzyme upon treatment with 8 mM C_{12}sulfate, while such exposure to 8 mM or 16 mM C_{14}sulfobetaine showed no change compared with the enzyme in buffer solution. Increase in negative ellipticity extrema of enzymes exposed to C_{12}sulfate has been previously noted with chymotrypsin and elastase (29) due to apparent increased α-helix content.

It has been suggested that at least an initial step in denaturation of invertase by C_{12}sulfate is ionic, at a pH level giving the enzyme a net positive charge (8). However, simple salt formation was ruled out (8) because the enzyme could not be renatured by raising the pH, following denaturation.

As shown here, ionic interactions do play a principal role in the denaturation of this enzyme, since activity lost by exposure to micellar concentrations of alkyl sulfate detergents can be restored by ion exchange chromatography.

In common with this anion exchanger used, the surfactant betaines contain a quaternary ammonium ion. However, surfactant betaines prevent loss of enzymatic activity due to alkyl sulfate surfactants only when the former are present prior to addition of the enzyme, but do not restore activity if the enzyme has been pretreated with these anionics.

Since only surface active betaines are effective as inhibitors, while nonsurface active homologs are not, it is reasonable to assume that protection afforded by these zwitterionics is due to comicellization.

Owing to the equimolar ratios of surface active betaines required, their effect may be resultant from the blocking of ionic interaction between sulfate anions of the alkyl sulfuric half esters with cationic (basic) residues of the enzyme. In micelles, the presence of sufficient betaine zwitterions in the Stern layer (30,31) may well prevent such binding (ionic interaction) which would otherwise occur.

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⁂Fatty Acid Amides and Anilides, Syntheses and Antimicrobial Properties[1]

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ABSTRACT

A series of fatty acid amides and anilides was prepared and a number of compounds in the series were found to be highly active against gram positive bacteria but ineffective against gram negative organisms. The N,N-dimethyl- and N,N-diethylamides of C_{12}-C_{14} fatty acids had minimal inhibitory concentration (MIC) values of 100 ppm or less. Substituted anilides of C_6-C_{11} fatty acids were active when the following groups were attached to the aromatic ring: 3,4-dichloro, 3-nitro, 4-nitro, 5-chloro-2-hydroxy, 4-chloro-3-nitro, and 2-hydroxy-5-nitro. Some of these compounds had a MIC value of 0.1 ppm. Significantly, the presence of soap did not reduce the activity of these bacteriostats, whereas polysorbate 80 at high concentrations deactivated the compounds.

INTRODUCTION

In spite of substantial research activity in the sanitizing field, the number of suitable antimicrobial agents used remains relatively small. Quaternary ammonium compounds, for example, are adversely affected by proteinaceous materials and hard water ions and are incompatible with anionic surfactants such as soap. Halogens, particularly chlorine, sodium hypochlorite, iodine and iodine complexes, are effective germicidal agents, but they lose their effectiveness because of volatility and instability due to their nonselective oxidation of organic matter. Halogens also attack textiles, some plastics and many metals.

Halogenated aromatic compounds, such as hexachlorophene, 3,4,4′-trichlorocarbanilide, 3,4,5-tribromosalicylanilide and 2,4,4′-trichloro-2-hydroxydiphenyl ether are fairly compatible with anionic surfactants and thus have been widely used in santizing cleaners, surgical scrub soaps, and deodorant toilet soaps. However, these antibacterial agents have drawbacks, and some have been banned because of toxicity, photosensitization and/or chemical instability. The fatty acid amides (1,2,3), on the other hand, are neutral derivatives of fatty acids and are compatible with soaps and anionic surfactants. Mitchell and Reid (4) made a series of fatty acid amides by passing ammonia gas through heated fatty acids, the method of commercial amide production. Similarly, D'Alelio and Reid (5) synthesized the series of N-methylamides. Earlier Robertson (6) characterized the N-phenylamides or anilides. Novak and coworkers (7,8) found that some N,N disubstituted amides, such as N,N-bis-(2-hydroxyethyl) lauramide, possessed antimicrobial activity. Kabara and coworkers (9) reported that N,N-dimethyllauramide is highly effective against *Staphylococcus aureus*. However, information in the literature concerning the antimicrobial activity of the fatty acid anilides is sparse (10), although mention is made of their effectiveness as herbicides (11) and as inhibitors of photosynthesis (12,13).

Beaver et al. (14) made a comprehensive study of the bacteriostatic properties of substituted ureas. These were remarkably specific in that activity was greatly enhanced or completely lost with slight changes in chemical structure. A similar systematic study of the fatty acid amides and anilides has not been reported in the literature.

The objective of our investigation was to develop chemical agents which ideally would be effective against both gram positive and gram negative bacteria but would not be rendered inactive by soaps and detergents. Fatty amides appeared to be promising agents, and their structures can be readily modified by: (a) varying the size of the fatty alkyl group; (b) placing one or two substituents on the amido nitrogen atom, and (c) changing the structure of the substituents on the nitrogen atom. The large number of substituted anilines commercially available thus made it feasible to synthesize a variety of fatty acid anilides.

A slight modification of the bacteriological screening technique of Beaver et al. (14) was used to determine antibacterial properties against five different microorganisms. Linfield et al. (15) reported that certain nonionic surfactants act as potentiating agents for antibacterial compounds. Accordingly, we also examined those fatty amides and anilides which displayed high antimicrobial activity for this potentiating effect.

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