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# Effect of rare earth cations on bactericidal activity of anionic surfactants

R.W. Berg and R.E. Zimmerer

*The Procter and Gamble Company, Cincinnati, OH, U.S.A.*

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## SUMMARY

Rare earth metal cations are antibacterially synergistic with anionic surfactants, yielding mixtures that have bactericidal activity against a variety of gram-positive and gram-negative bacteria at minimum concentrations ranging from 16 to 125 μg/ml. Uptake of surfactant by *Escherichia coli* increases in the presence of lanthanum, suggesting that the role of rare earth metal cations is to reduce the net negative surface charge on the bacteria, thereby increasing the affinity between the negatively charged surfactant and the bacterial surface.

## INTRODUCTION

Surface-active agents are believed to kill susceptible bacteria by damaging cytoplasmic membranes. Anionic surfactants are moderately active against gram-positive organisms, but have little or no activity against gram-negatives, presumably because the outer membranes of gram-negative bacteria do not allow penetration by the surfactant to the cytoplasmic membrane.

Voss [12] reported that the bactericidal effectiveness of several anionic surfactants against *Staphylococcus aureus* is increased in the presence of low concentrations of divalent cations, especially alkaline earths and metals of group IIB of the periodic table. However, these combinations did not result in increased activity against *Escherichia coli*.

This paper describes the broad spectrum antibacterial activity and proposed mechanism of action of rare earth (lanthanides) metal salts of anionic surfactants.

Correspondence: Dr. R.W. Berg, The Procter and Gamble Company, Miami Valley Laboratories, P.O. Box 39175, Cincinnati, OH 45247, U.S.A.



## MATERIALS AND METHODS

### *Source of chemicals and microorganisms*

Seven anionic surfactants were studied. Sodium 4-(1′-methylnonyl)benzenesulfonate (2-$C_{10}$LAS) (100% surfactant), sodium 2-$C_{12}$LAS (100% surfactant), sodium coconut monoglyceride sulfonate (95% surfactant), and sodium lauryl trioxyethylene sulfate (55.8% surfactant, remainder $Na_2SO_4$) were synthesized at the Procter and Gamble Company. Sodium decyl sulfate and sodium dodecyl sulfate were obtained as pure compounds from Eastman Kodak Company. Sodium dioctylsulfosuccinate (85% surfactant) was obtained from American Cyanamide. Rare earth metal salts (>99.9% pure) were obtained from Alfa Products. Dodecyltrimethylammonium chloride was purchased from Eastman Kodak, and all bacterial cultures were obtained from the American Type Culture Collection.

*Antibacterial assays*

Aqueous test solutions were diluted serially in sterile distilled water to achieve appropriate concentrations in a final volume of 5 ml. Test organisms were grown for 24 h at 35°C in Trypticase Soy Broth (TSB), a 1:100 dilution in sterile distilled water was prepared, and 0.05 ml of this diluted culture was used to inoculate the 5 ml test solution. Inoculated test solutions (containing about $10^5$ organisms/ml) were incubated for 10 min at 37°C, and survivors were enumerated by serial tube dilution in TSB or by standard plate count on Trypticase Soy Agar. The minimum bactericidal concentration was defined as the lowest concentration of test material that resulted in no recoverable organisms.

*Adsorption of 2-$C_{10}$LAS on E. coli*

An 18 h TSB culture of *E. coli* ATCC 11775 was washed by repeated (3 ×) centrifugation and resuspension in distilled water. The bacteria were finally suspended in solutions having concentrations of $3 \times 10^{-6}$ M $^{14}$C-(2-$C_{10}$LAS) containing $LaCl_3$ ranging from $10^{-5}$ to $10^{-3}$ M, or $MgCl_2$ ranging from $10^{-1}$ to 1.0 M. The organisms were present at levels of approximately $10^6$/ml. Following incubation at 25°C for 30 min, the cells were harvested by centrifugation, and the concentration of 2-$C_{10}$LAS in the pellet and supernatant fractions was determined by liquid scintillation counting on a Packard model 2450 Spectrometer. Uptake of LAS was calculated as the percent of the original concentration removed from solution by the bacteria.

## RESULTS

The minimum bactericidal concentrations of several anionic surfactants alone and in the presence of $LaCl_3$ are shown in Table 1. In general, the surfactants were moderately active against the gram-positive *S. aureus*, and inactive against the gram-negative *E. coli*. However, when the surfactants were combined with $LaCl_3$, the antibacterial activity against both microorganisms was greatly increased. Moreover, the combination exhibited efficacy comparable to that of quaternary ammonium disinfectants [8].

The bactericidal activity of 2-$C_{10}$LAS against *E. coli* in the presence of several rare earth chlorides is shown in Table 2. The metal salts alone had little or no antibacterial activity at the concentrations tested, but all exhibited a potent synergism with the surfactant.

The minimum bactericidal concentrations of the combination of 2-$C_{10}$LAS with $LaCl_3$ against a variety of gram-positive and gram-negative bacteria is shown in Table 3. The mixture was broadly active against these organisms at minimum concentrations ranging from 10 to 125 $\mu$g/ml.

It seemed probable that the increased kill of *E. coli* by 2-$C_{10}$LAS in the presence of $LaCl_3$ was due to increased adsorption of the anionic surfactant by the negatively charged cells. Therefore, the adsorption of 2-$C_{10}$LAS was determined in the presence of various concentrations of $LaCl_3$. If the effect of $LaCl_3$ was indeed to reduce the net surface charge, divalent cations such as $Mg^{2+}$ at higher concentrations should also exhibit antibacterial synergism with the surfactant. Uptake of 2-$C_{10}$LAS by *E. coli* and cell death as a function of added lanthanum or magnesium ions is shown in Fig. 1. Both metal ions increased adsorption of the surfactant and acted as antibacterial synergists, but about four orders of magnitude more magnesium than lantha..um was required for the same effect.

Quaternary ammonium surfactants are effective antibacterial agents in part because of the attraction between the positive center in the molecule and

Table 1

Minimum bactericidal concentrations of anionic surfactants with and without added $LaCl_3$

| Surfactant | Minimum bactericidal conc. ($\mu$g/ml) | | | |
|---|---|---|---|---|
| | *E. coli* ATCC 11775 | | *S. aureus* ATCC 6538 | |
| | surfactant alone | surfactant + $LaCl_3$[a] | surfactant alone | surfactant + $LaCl_3$[a] |
| Sodium 2-$C_{10}$LAS | >2000 | 16 | 2000 | 32 |
| Sodium 2-$C_{12}$LAS | >2000 | 32 | 125 | 32 |
| Sodium decyl sulfate | >2000 | 63 | 2000 | 250 |
| Sodium dodecyl sulfate | >2000 | 125 | 500 | 32 |
| Sodium lauryl trioxyethylene sulfate | >2000 | 32 | >2000 | 63 |
| Sodium dioctylsulfosuccinate | >2000 | 32 | 1000 | 63 |
| Sodium coconut monoglyceride sulfonate | >2000 | 16 | >2000 | 63 |

[a] $LaCl_3$ added to surfactant solution at 1:3 molar ratio. Concentration expressed as La(Surfactant)$_3$. The minimum bactericidal concentration of $LaCl_3$ under these conditions is >3500 ppm (0.01 M).

negatively charged bacteria. If the role of rare earth cations is to reduce the net negative charge on or in the bacterial surface, one would expect lanthanum to decrease the antibacterial efficacy of quaternary ammonium surfactants. The data in Table 4 demonstrate that this is indeed the case.

Table 2

Minimum bactericidal concentrations of rare earth metal salts combined with 2-$C_{10}$LAS

| Metal salt | Minimum bactericidal conc.[a] | |
|---|---|---|
| | Metal salt alone ($\mu$g/ml) | 2-$C_{10}$LAS ($\mu$g/ml) in presence of metal salt[b] |
| None | – | >2000 |
| $LaCl_3$ | >2500 | 16 |
| $DyCl_3$ | >2500 | 10 |
| $NdCl_3$ | >2500 | 16 |
| $PrCl_3$ | >2500 | 3 |
| $YbCl_3$ | >2500 | 3 |

[a] *E. coli* ATCC 11775

[b] 3:1 molar ratio, surfactant:lanthanide

Table 3

Minimum bactericidal concentration of lanthanum/LAS against representative gram-positive and gram-negative bacteria

| Organism | Minimum bactericidal conc. ($\mu$g/ml)[a] |
|---|---|
| *Staphylococcus aureus* ATCC 6538 | 32 |
| *Staphylococcus epidermidis* ATCC 14990 | 32 |
| *Micrococcus luteus* ATCC 4690 | 125 |
| *Streptococcus faecalis* ATCC 6569 | 32 |
| *Escherichia coli* ATCC 11775 | 16 |
| *Pseudomonas aeruginosa* ATCC 15442 | 16 |
| *Klebsiella pneumoniae* ATCC 4352 | 125 |
| *Proteus vulgaris* ATCC 13315 | 32 |

[a] as La(2-$C_{10}$LAS)$_3$

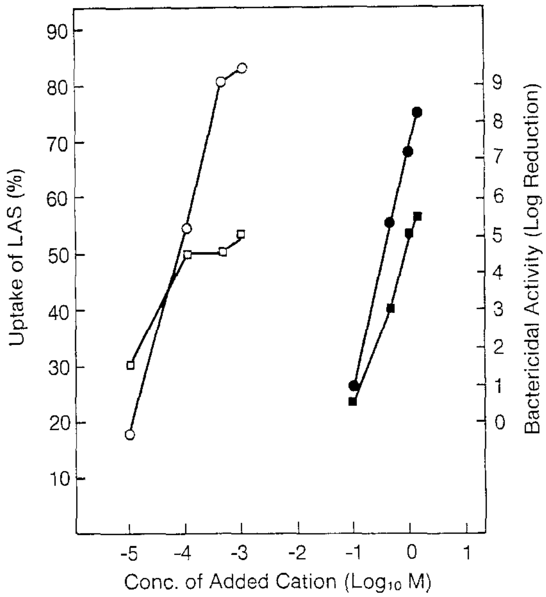


Fig. 1. Effect of cations on bactericidal activity and uptake of 2-$C_{10}$LAS by *E. coli*. ○ = LAS uptake in the presence of $La^{3+}$; ● = LAS uptake in the presence of $Mg^{2+}$; □ = bactericidal activity of LAS in the presence of $La^{3+}$; ■ = bactericidal activity of LAS in the presence of $Mg^{2+}$.

## DISCUSSION

Several hypotheses have been advanced to explain the bactericidal action of surface-active agents, including general denaturation of cell proteins, specific inactivation of enzymes, and the disorganization of cell permeability barriers. The relative merits of these hypotheses have been reviewed by Newton [5], and the accumulated data suggest that treatment of bacteria with certain surfactants results in a rapid disorganization of the cytoplasmic membrane. Moreover, the key to efficacy of surfactants against bacteria appears to be the ability to reach the cytoplasmic membrane. In order to reach this site, they must penetrate the outer layers of the cells, layers which are vastly different in gram-positive and gram-negative bacteria. Gram-positive organisms have a relatively porous peptidoglycan cell wall which has a molecular exclusion limit of up to 100 000 amu [9]. In addition to a similar though somewhat thinner cell wall, gram-negative bacteria possess a lipoprotein outer membrane that confers additional barrier properties on the cells. This membrane contains pores which act as molecular sieves with exclusion limits of 500–700 amu for saccharides [1] and peptides [7]. Inouye [4] has suggested that these pores are water-filled and lined with acidic amino acids, the ionized carboxyl groups of which result in a negatively charged interior at physiological pH. Anionic surfactants presumably are not effective against gram-negative bacteria because of the charge repulsion that exists between the surfactant and the anionic bacterial surface. Moreover, negatively charged pores may be of ecological advantage to *E. coli*, which must exclude anionic bile salts from its natural habitat [6]. A decrease in the net negative charge on bacteria should result in an increase in uptake, penetration, and antibacterial efficacy of anionic surfactants. This can be accomplished by lowering the pH of the environment. Gram-negative bacteria have isoelectric points in the range of pH 4–5 [11], and acidic anionic surfactant solutions are indeed active against these organisms [2].

Table 4

Antibacterial efficacy of a cationic surfactant in the presence of lanthanum

| Material tested | $Log_{10}$ survivors[a]/ml |
|---|---|
| 100 ppm $C_{12}$TMAC[b] | 0 |
| 100 ppm $C_{12}$TMAC + 0.1 mM $La^{3+}$ | 4 |
| 0.1 mM $La^{3+}$ | 6 |
| $H_2O$ control | 7 |

[a] *E. coli* ATCC 11775
[b] $C_{12}H_{25}N^+(CH_3)_3$, $Cl^-$

Another way of reducing the charge on bacteria is by the addition of appropriate cations. Lanthanides are particularly efficient because they have high charge densities, and exist substantially as unhydrolyzed trivalent ions at physiological pH. This is reflected by relatively low log $K$ values for association of $La^{3+}$ with hydroxide ion compared with other multivalent ions such as $Al^{3+}$ and $Fe^{3+}$ [10]. Moreover, Falk [3] reported reversal of the surface charge of *E. coli* by lanthanum ion as measured by changes in electrophoretic mobility. While many divalent ions such as $Ca^{2+}$ and $Mg^{2+}$ are relatively unhydrolyzed in solution, they do not have high enough charge densities to be effective at low concentrations. The combination of an anionic surfactant with a lanthanide cation therefore represents a unique and potentially useful example of antibacterial synergism.

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