Antimicrobial Activity of Betaine Esters, Quaternary Ammonium Amphiphiles Which Spontaneously Hydrolyze into Nontoxic Components

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Received 27 April 1990/Accepted 23 July 1990

A series of quaternary ammonium compounds that are esters of betaine and fatty alcohols with hydrocarbon chain lengths of 10 to 18 carbon atoms were tested with respect to antimicrobial activities and rates of hydrolysis. When the tetradeacly derivative was tested against some selected microorganisms, the killing effect was comparable to that of the stable quaternary ammonium compound cetyltrimethylammonium bromide. At higher pH values, both the antimicrobial effect and the rate of hydrolysis of the esters increased. However, whereas at pH 6 greater than 99.99% killing of Salmonella typhimurium was achieved with 5 μg/ml in 3 min, the rate of hydrolysis was less than 20% in 18 h. At pH 7, a similar killing effect was achieved in 2 min and 50% hydrolysis occurred in ca. 5 h. Thus, it is possible to exploit the rapid microbial effect of the compounds before they hydrolyze. The rate of hydrolysis was reduced by the presence of salt. The bactericidal effect of the betaine esters increased with the length of the hydrocarbon chain of the fatty alcohol moiety up to 18 carbon atoms. Since the hydrolysis products are normal human metabolites, the hydrolysis property may extend the use of these quaternary ammonium compounds as disinfectants and antiseptics for food and body surfaces.

The membrane-disruptive and antimicrobial activities of cationic surfactants are well recognized. These agents are often active against a broad range of bacteria and other cells and can also inactive certain viruses (16, 29). Because of their high affinity for biological membranes, these agents show a low selectivity and can be damaging to a variety of mammalian cells (17, 23, 24).

Since the time needed to kill microorganisms with cationic surfactants is usually short, it could be expected that side effects in the host might be decreased by the use of substances that are subject to hydrolytic degradation. However, the lifetime of the compounds must be sufficiently long to allow proper inactivation of the undesired microorganisms. The products obtained in the degradation steps should also be significantly less toxic than the original compound and should ideally constitute normal metabolites of the host.

To explore the possible use of degradable cationic surfactants, we studied a series of amphiphilic betaine esters (Fig. 1). Although the increased rate of base-catalyzed hydrolysis of lower esters of this structure has been investigated and is understood to be caused by an inductive effect from the positive charge (5, 26), this property does not seem to have been considered in connection with biological (10, 27) or recent surface chemical (4, 25) studies of compounds of this type. In the present paper, we describe the synthesis of some esters between betaine (trimethylglycine) and long-chain fatty alcohols, their antimicrobial activities, and their rates of hydrolysis.

MATERIALS AND METHODS

Compounds investigated. The betaine esters were obtained as chlorides by chloroacetylation of the corresponding long-chain alcohol, principally as described by Holden (14), followed by quaternization with trimethylamine. The method is exemplified by the synthesis of tetradeacly betainate (B-14). Tetradeacly (10.0 g, 46.7 mmol) was dissolved in 50 ml of dry dichloromethane at 30°C, chloroacetyl chloride (5.37 g, 47.6 mmol) was added over a period of 15 min, and the mixture was kept at 30°C for 1 h and washed twice with a 5% solution of sodium hydrogen carbonate and twice with distilled water. After drying and evaporation of the dichloromethane, the tetradeacly chloroacetate was formed directly with trimethylamine. The product (11.9 g, 40.9 mmol) was dissolved in 20 ml of aceton at 50°C, dry trimethylamine (2.9 g, 49.1 mmol) in 20 ml of aceton was added over a period of 20 min, and the mixture was kept at 50°C for 3 h and allowed to cool, yielding a crystal mass which was isolated on a glass filter and washed with acetone. The product was recrystallized from ethyl acetate.

Nuclear magnetic resonance (NMR) spectra were recorded with the use of a Bruker model WH 270 Fourier transform spectrometer. The samples were dissolved in deuteriochloroform, with tetramethylsilane as an internal standard. Thin-layer chromatography was performed on Merck 60 F254 silica plates with chloroform-methanol-water-acetic acid (60:35:8:11.4 [vol/vol]) as the mobile phase, and spots were visualized with iodine vapor or charring reagent (5% sulfuric acid in ethanol).

Microorganisms and culture conditions. The microbicidal activity of B-14 was tested on Escherichia coli ATCC 25922, Salmonella typhimurium 395MS (an S [smooth] strain described in detail earlier [9]), Pseudomonas aeruginosa CCUG (University of Göteborg Culture Collection) 4625 (ATCC 23389), Bacillus megaterium CCUG 1817 (ATCC 1458), and Candida albicans (clinical isolate) with the stable structural analog cetyltrimethylammonium bromide (CTAB; approximately 99% pure; Sigma Chemical Co., St. Louis, Mo.) as a reference substance. For further studies on the bactericidal activity of B-14 and related compounds, S. typhimurium 395MS was used. The bacteria were grown in 10 ml of glucose broth (beef extract, 5 g; peptone, 10 g; disodium hydrogen phosphate dihydrate, 0.6 g; sodium

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Antimicrobial effect of betaine esters as compared with that of CTAB. A number of tests were performed to compare the antimicrobial effect of the betaine esters with that of the stable cationic surfactant CTAB. Microorganisms were exposed to different concentrations of B-14 or CTAB at 30°C (Fig. 3). All the bacteria tested, that is, B. megaterium, P. aeruginosa, and S. typhimurium, were killed completely (>3.5 log inactivation in 10 min). For B. megaterium, 2 μM was sufficient, whereas 10 μM was needed for P. aeruginosa and S. typhimurium. For killing of C. albicans, 50 μM for 30 min was sufficient. The other concentrations yielded corresponding inactivation rates (data not shown). Generally, CTAB was slightly more effective than B-14. Antimicrobial effect of betaine esters as a function of chain length. To find the optimal chain length of the betaine esters with respect to bactericidal effect, we synthesized a series of esters with 10 to 18 carbon atoms in the alky1 chain (B-10 to B-18) and tested them against S. typhimurium 395MS. In 10 mM sodium phosphate buffer at pH 7.0 and 30°C, B-18 showed the highest activity whereas derivatives B-10 and B-12 did not affect the viability appreciably at 20 μM concentrations (Fig. 4).

RESULTS

Product characterization. The substances were obtained as white glistening crystals after recrystallization from ethyl acetate. The yields were from 65 to 84%, calculated on the amount of fatty alcohol used in the first step of the synthesis. 1H NMR spectra were in complete agreement with the expected structures and showed essentially no contaminants. Figure 2 shows the 1H NMR spectrum of B-14. Melting points were not well defined and were therefore of limited value. Very different melting points are given for the chlorides of dodecyl betaine (B-12) and hexadecyl betaine (B-16) in the literature (4, 10, 21), with values ranging from 57 to 215°C and from 75 to 215°C, respectively. These discrepancies were probably due to the ability of the compounds to form liquid crystalline mesophases and an inadequate characterization of their melting behavior. Thin-layer chromatography revealed no contaminants.

Antimicrobial activity of betaine esters. Microbical activity was generally tested in 50-ml sterile Erlenmeyer flasks to which 24.5-ml portions of sterile 10 mM buffer (see figure legends for compositions) were added, with the temperature adjusted to 30°C, in a shaking water bath. To one flask was added 250 μl of washed cell suspension and 250 μl of a stock solution of the test compound (dissolved in sterile 1 mM hydrochloric acid to minimize hydrolysis), and the flask was placed in the shaker. After appropriate lengths of time, a 0.5-ml portion of the suspension was transferred to 4.5 ml of Letheen broth (Thiotone peptone [BBL Microbiology Systems, Cockeysville, Md.], 10.0 g; beef extract, 5.0 g; lecithin, 0.7 g; polysorbate 80, 5.0 g; sodium chloride, 5.0 g; distilled water, 1,000 ml). This broth inactivates quaternary ammonium germicides (28) and is recommended as a neutralizing diluent for the evaluation of disinfectants containing cationic surfactants (3). Further dilution of the sample was also made with Letheen broth. From the dilutions, 50-μl samples were spread onto glucose agar plates (glucose broth plus 1.5% agar) with an automatic spread plate system (model D Spiral Plater; Spiral Systems Instruments, Inc., Bethesda, Md.). The plates were incubated at 37°C for 18 h, and the number of colonies was counted. Controls were prepared and treated as described above, except that 250 μl of sterile 1 mM hydrochloric acid without test compound was added.

The bactericidal activity of B-14 at different pHs was tested similarly at a fourfold volume. The following 10 mM buffers were used: sodium citrate at pH 4.0, 5.0, and 6.0; sodium phosphate at pH 6.0, 7.0, and 8.0; Tris hydrochloride at pH 9.0; and glycine at pH 9.0. The buffers were chosen to achieve adequate buffering capacity. At the same pHs, similar killing effects were obtained in the different salt solutions. No killing was observed in the absence of B-14.

Hydrolysis rate determination. Ester hydrolysis was effected in 50 mM buffers at different pHs, and the liberated alcohol was determined by gas chromatography after extraction into hexane. The hydrolysis procedure was as follows.

Aliquots (50 μl) of different buffer solutions were transferred to a series of 100-ml glass flasks with screw caps and placed in a thermostat. After temperature equilibration, 400 μl of a solution of the ester in ethanol (10 mg/ml) was added to each flask and mixed, and the mixture was immediately distributed in 1-ml aliquots into small glass tubes with screw caps and placed in the thermostat. After different lengths of time, these 1-ml volumes of the reaction mixture were extracted with 200 μl of hexane containing 0.20 mg of hexadecanol per ml (used as an internal standard). After centrifugation (2,500 × g, 10 min), the hexane phase was transferred to a small gas chromatography vial which was sealed, and the hexane solution was directly injected into the gas chromatograph for analysis. An Antek model 460 instrument equipped with a flame ionization detector and a 6-ft (ca. 180-cm) packed glass column containing 3% SP-2100 on 80/100 Supelcoport (Supelco Inc., Bellefonte, Pa.) was used. The flow rate of the nitrogen carrier gas was 20 ml/min. Immediately after injection of 1.5 μl of the hexane solution, the column temperature was raised from 110 to 180°C at a rate of 10°C/min. The retention times obtained were 5 and 7.5 min for tetra- and hexadecanol, respectively. The reproducibility was estimated by repeated coextractions of a known concentration of hexadecanol. The coefficient of variation was 5.8% (n = 66).

FIG. 1. Chemical structure and hydrolysis of the compounds investigated.
Bactericidal activity of tetradecl betaine as a function of pH. Since the rate of hydrolysis of B-14 is very pH dependent (see below), the bactericidal activity was investigated as a function of pH. The killing rate was considerably reduced at higher hydrogen ion concentrations (Fig. 5). In the presence of 5 μg of B-14 per ml (14 μM), the killing rate gradually decreased as the pH was reduced stepwise from 8 to 5; at pH 4 it was reduced to <1 log over the 10-min period. Controls did not show any killing in the different buffers in the absence of betaine esters.

Rates of hydrolysis. A dramatically increased rate of hydrolysis with increasing pH of the reaction medium was found for B-14 at 30°C (Fig. 6). The half-life of ca. 5 h obtained at pH 7.0 decreased to less than 1 h at pH 8.0. At pH 7.0, a decrease in the temperature from 30 to 25°C approximately doubled the half-life.

A very pronounced effect of ionic strength on the rate of hydrolysis was also found (Fig. 7). While hydrolysis in 50 mM buffer at pH 7.9 was almost complete (95%) after 2 h, it was only 4% when 1 M sodium chloride was added to the buffer.

DISCUSSION

The interaction between cationic surfactants and microbial cells is not understood in detail. It seems generally accepted, however, that lipid bilayer structures of cell membranes are principal targets for this class of compounds. In the process of binding, the hydrocarbon tail of the cationic amphiphilic substance becomes intercalated into the hydrophobic interior of the microbial membrane, and the cationic polar head group participates in charge interactions with neighboring surface structures (18). Accordingly, since the betaine portion and its quaternary ammonium moiety are identical in our series of long-chain alkyl esters, the differences in antimicrobial activities between the esters are dependent on the length of the lipophilic moiety. We found the highest bactericidal activity for the octadecyl (B-18) and the hexadecyl (B-16) derivatives, the compounds with the longest chains tested. B-14, B-16, and B-18 were all highly effective at concentrations below 10 mM, while the decyl (B-10) and the dodecyl (B-12) derivatives lacked activity at concentrations up to 20 μM under the experimental conditions used (Fig. 4). As for stable quaternary ammonium compounds, the initial site of interaction of the betaine esters is probably the lipid bilayer of the outer membrane (13). Furthermore, these substances cause leakage of cytoplasmic
compounds, indicating that the plasma membrane is also affected (12, 15). The phospholipids of both types of membranes contain fatty acids, mainly C16 and C18 (8), and there is a rapid exchange between the phospholipids of the outer and inner membranes (22). In the lipopolysaccharide of *Salmonella* strains, the 3-hydroxytetradecanoic acid residues, which are amide linked to the glucosamine moieties of lipid A, are 3-O-acylated by C12 and C16 saturated fatty acids (32), allowing a hydrocarbon chain length in the outer cell membrane of at least 18 carbon atoms. Thus, the high bactericidal activity of the C16 and C18 betaine esters may be due to the facts that both the outer and the plasma membrane lipid bilayers may accommodate the entire hydrocarbon chain length of these betaine esters and that longer hydrophobic chains have a greater hydrophobic effect (31). Charge interactions between quaternary nitrogen groups in betaine esters and phosphate groups in phospholipids and lipopolysaccharide may contribute to complex formation (18). The higher antibacterial activity of octadecyl quaternary ammonium compounds was not shown in earlier comparative tests of series of substances with different hydrocarbon chain lengths and other chemical structures adjacent to the quaternary nitrogen (19). This difference need not be a consequence of the different kinds of quaternary ammonium compounds but may be due to the temperature, suspension medium, and experimental set-up chosen, since the complex formation is far from specific and since hydrophobic and charged compounds in the suspension medium may affect antimicrobial potency. Experiments are in progress which address this question. The shapes of the inactivation curves by B-16 and B-18 (concave upwards) may indicate that as the total concentration of the esters is increased, the proportion available for bactericidal action is decreased. This result could be due to factors such as the lower water solubility of these derivatives as compared with that of B-14 or a greater tendency of these more hydrophobic compounds to bind in excessive amounts to already inactivated cells or to form inactive aggregates with compounds released from the cells.

**FIG. 4.** Relationship between length of the alkyl chain and bactericidal activity of betaine against *S. typhimurium* 395MS in 10 mM sodium phosphate buffer (pH 7.0) at 30°C. The length of contact was 3 min. Each point represents the mean of two separate experiments, and the duplicate values are connected by a vertical bar, most often covered by the symbol. The limit of detection of the procedure is indicated by the dotted line. Symbols: ▲, dodecyl betaine (B-12); Δ, tetradecyl betaine (B-14); ●, hexadecyl betaine (B-16); ○, octadecyl betaine (B-18). Decyl betaine (B-10) showed the same result as B-12.

**FIG. 5.** Effect of pH on the bactericidal activity of 5 μg of tetradecyl betaine (B-14) per ml (14 μM) at 30°C against *S. typhimurium* 395MS. Each point represents the mean of two separate experiments, and the duplicate values are connected by a vertical bar, most often covered by the symbol. The limit of detection of the procedure is indicated by the dotted line. Symbols: ▲, pH 4.0; ○, pH 5.0; △, pH 6.0; Δ, pH 7.0; ■, pH 8.0. At pH 9.0 as well as at pH 8.0 complete killing was achieved in 1 min.

**FIG. 6.** Dependence on pH of the rate of hydrolysis of tetradecyl betaine (B-14) at pH 3 (●), pH 5 (△), pH 6 (■), pH 7 (▼), pH 8 (▲), and pH 9 (○), all at 30°C, except that the hydrolysis rate at pH 7 was also tested at 25°C (▼). Each point represents one determination.

**FIG. 7.** Effect of sodium chloride on the rate of hydrolysis of tetradecyl betaine (B-14) at 30°C in 50 mM phosphate buffer (pH 7.9). Sodium chloride was used at 1 M (△), 0.5 M (▲), and 0.1 M (○); ●, control. Each point represents one determination.
The bactericidal activity of B-14 at 5 μg/ml decreased when the pH of the medium was reduced. Nearly no killing effect was seen at pH 4 (Fig. 5). In general, the antimicrobial activity of amphiphilic quaternary ammonium compounds is less pronounced at an acidic pH (28), and decreased binding of CTAB to yeast cells around pH 5 has been reported (11). Furthermore, the adsorption of dodecyl trimethylammonium bromide to artificial dipalmitoylphosphatidylethanolamine membranes is decreased by an increase in the hydrogen ion concentration (20). The decreased adsorption and killing effect of quaternary ammonium compounds at a lower pH may be a consequence of suppressed ionization of the phosphate groups of the phospholipids and lipopolysaccharide of the outer and plasma membranes at a lower pH, leading to decreased electrostatic attraction of the cationic amphiphile.

The hydrolytic instability of the ester bond is caused by the positive charge on the nitrogen atom, which reduces the free activation energy of alkaline hydrolysis. Therefore, the ester bond hydrolyzes in neutral media at a substantial rate even at room temperature. Conversely, the rate of acid hydrolysis is slower than in uncharged esters (5), generating the extreme pH dependence of the rate of hydrolysis (Fig. 6). However, killing of bacteria is more rapid at pH 9 than at pH 5 (Fig. 5), probably because of the increased electrostatic attraction of the betaine ester to the bacterial cell at a basic pH.

Our results indicate that the incorporation of an ester function into the molecule does not significantly influence the activity against microorganisms that are sensitive to cationic surfactants, as long as the ester bond is intact. When the cationic surfactants were used even under conditions that promoted a high rate of hydrolysis, the bactericidal effect was comparable to that obtained with an analogous cationic surfactant lacking the ester group, CTAB.

Cationic surfactants are of interest because of their membrane-disruptive and rapid antimicrobial activities (1, 17, 18). Inclusion in liposomes has been tried in vitro to modify and extend their use (24, 30). However, low selectivity in the toxicity of traditional cationic surfactants may restrict their application, particularly with respect to therapeutic purposes. Side effects due to the persistence of biologically active compounds may be reduced through the design of analogs containing hydrolyzable bonds (2, 6). This strategy was previously applied to cationic surfactants by Bodor et al. (7) with a series of esters between quaternized α-aminoalcohols and fatty acids which were termed soft antimicrobial agents. The amphiphilic betaine esters described above will be another group of such agents.

Because the time needed for microbial killing is short, a reduction of the lifetime of the ester by hydrolysis should allow effective disinfection and antiseptics with reduced toxic effects. It may be hypothesized that the killing of microbes suspended as single cells is much more rapid than is damage to mammalian cells at body surfaces like skin and mucous membranes, where antisepsics are often used, since the latter cells are part of tissue aggregates covered by a protective coat which reduces accessibility. Such a mechanism may lead to new applications for the betaine esters, in situations in which stable quaternary ammonium compounds have been avoided because of their general membrane-damaging and toxic effects. The betaine esters may also have less of an effect on the environment.

ACKNOWLEDGMENTS
This work was supported by grants from the National Swedish Board for Technical Development (grant 732-88-01173) and Berol Nobel AB.

NMR facilities were kindly placed at our disposal at the Department of Organic Chemistry, University of Göteborg, which we gratefully acknowledge.

LITERATURE CITED
1. Ancelin, M. L., and H. J. Vial. 1986. Quaternary ammonium compounds efficiently inhibit Plasmodium falciparum growth in vitro by impairment of choline transport. Antimicrob. Agents Chemother. 29:814–820.
2. Arlész, E. J., and A. Simónis. 1974. Design of bioactive compounds. Top. Curr. Chem. 52:1–61.
3. Association of Official Analytical Chemists. 1984. Disinfectants, p. 65–77. In Official methods of analysis, 14th ed. Association of Official Analytical Chemists, Arlington, Va.
4. Beger, V. J., R. Jacobi, and R. Köhler. 1983. Mehrfunktionelle N-Tenside. VI. Synthesen und oberflächenaktive Eigenschaften von quartenierten Dimethylaminoessigsäurederivaten. Tenside Deterg. 20:169–172.
5. Bell, R. P., and F. J. Lindars. 1954. Kinetics of the acid and alkaline hydrolysis of ethoxycarbonylmethyltrimethylammonium chloride. J. Chem. Soc. 1954:4601–4604.
6. Bodor, N. 1984. Soft drugs: principles and methods for the design of safe drugs. Med. Res. Rev. 4:449–469.
7. Bodor, N., J. J. Kaminski, and S. Selk. 1980. Soft drugs. 1. Labile quaternary ammonium salts as soft antimicrobials. J. Med. Chem. 23:469–474.
8. Cronan, J. E., and C. O. Rock. 1987. Biosynthesis of membrane lipids, p. 474–497. In F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), Escherichia coli and Salmonella typhimurium: cellular and molecular biology, vol. 1. American Society for Microbiology, Washington, D.C.
9. Edebo, L., E. Kihlström, K.-E. Magnusson, and O. Stendahl. 1980. The hydrophobic effect and charge effects in the adhesion of enterobacteria to animal cell surfaces and the influence of antibodies of different immunoglobulin classes, p. 65–101. In A. S. G. Curtis and J. D. Pitts (ed.), Cell adhesion and motility. 3rd. Symposium of the British Society for Cell Biology. Cambridge University Press, Cambridge.
10. Epshtein, A. E., V. E. Limanov, M. Y. Telegin, E. K. Skvortsova, and T. I. Maksimova. 1980. Bactericidal quaternary ammonium salts derived from monocloroacetate esters. Pharm. Chem. J. (Engl. Trans. Khim. Farm. Zh. 14:23–26) 1981:292–295.
11. Fujita, T., and S. Koga. 1966. The binding of a cationic detergent by yeast cells in relation to its germicidal action. J. Gen. Appl. Microbiol. 12:229–237.
12. Hamilton, W. A. 1970. The mode of action of membrane-active antibacterial agents. FEBS Symp. 20:71–79.
13. Hancock, R. E. W. 1984. Alterations in outer membrane permeability. Annu. Rev. Microbiol. 38:237–264.
14. Holden, D. A. 1983. Synthesis and spreading behavior of some reactive derivatives of long-chain alcohols and carboxylic acids. Can. J. Chem. 62:574–579.
15. Hugo, W. B. 1982. Disinfection mechanisms, p. 158–185. In A. D. Russel, W. B. Hugo, and G. A. J. Ayliffe (ed.), Principles and practice of disinfection, preservation and sterilisation. Blackwell Scientific Publications, Ltd., Oxford.
16. Hugo, W. B., and A. D. Russel. 1982. Types of antimicrobial agents, p. 8–106. In A. D. Russel, W. B. Hugo, and G. A. J. Ayliffe (ed.), Principles and practice of disinfection, preservation and sterilisation. Blackwell Scientific Publications, Ltd., Oxford.
17. Isomaa, B., H. Hägerstrand, G. Pautero, and A. C. Engblom. 1986. Permeability alterations and antihaemolysis induced by amphiphiles in human erythrocytes. Biochim. Biophys. Acta 860:510–524.
18. Jawetz, E., J. L. Melnick, E. A. Adelberg, G. F. Brooks, J. S. Butel, and L. N. Ornston. 1989. Medical microbiology, p. 46.
Prentice-Hall, London.

19. Linfield, W. M. 1970. Straight-chain alkylammonium compounds, p. 9–70. In E. Jungermann (ed.), Cationic surfactants. Marcel Dekker, Inc., New York.

20. Matsumura, H., M. Ivamoto, and K. Furusawa. 1986. Adsorption of cationic surfactants on phospholipid membranes and its contributions to membrane-surface potential. Bull. Chem. Soc. Jpn. 59:1533–1537.

21. Métayer, M., and J. Jacob. 1952. Préparation et propriétés pharmacodynamiques de quelques esters de la bétaine. Ann. Pharm. Fr. 10:435–440.

22. Nikaido, H., and M. Vaara. 1987. Outer membrane, p. 7–21. In F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), Escherichia coli and Salmonella typhimurium: cellular and molecular biology, vol. 1. American Society for Microbiology, Washington, D.C.

23. Paatero, G. I. L., D. L. Brown, and P. D. Waterhouse. 1986. Inhibition of surface immunoglobulin capping on mouse splenic lymphocytes by cetyltrimethylammonium bromide. Cell. Mol. Biol. 32:79–85.

24. Pinnaduwage, P., L. Schmitt, and L. Huang. 1989. Use of a quaternary ammonium detergent in liposome mediated DNA transfection of mouse L-cells. Biochim. Biophys. Acta 985:33–37.

25. Rózycka-Roszak, B., S. Przestalski, and S. Witek. 1988. Calorimetric studies of the micellization of some amphiphilic betaine ester derivatives. J. Colloid Interface Sci. 125:80–85.

26. Robson Wright, M. 1968. Arrhenius parameters for the alkaline hydrolysis of esters in aqueous solution. III. Methyl betaine methyl ester. J. Chem. Soc. Sect. B 1968:548–550.

27. Rucka, M., M. Oswiecimska, and S. Witek. 1983. New biocides for cooling water treatment. III. Quaternary ammonium salts derivatives of glycine esters. Environ. Prot. Eng. 9:25–31.

28. Russel, A. D. 1982. Factors influencing the efficacy of antimicrobial agents, p. 107–137. In A. D. Russel, W. B. Hugo, and G. A. J. Ayliffe (ed.), Principles and practice of disinfection, preservation and sterilisation. Blackwell Scientific Publications, Ltd., Oxford.

29. Sands, I. A. 1986. Virucidal activity of cetyltrimethylammonium bromide below the critical micelle concentration. FEMS Microbiol. Lett. 36:261–263.

30. Tachibana, H., E. Yoshibara, Y. Kaneda, and T. Nakae. 1988. In vitro lysis of the bloodstream forms of Trypanosoma brucei gambiense by stearylamine-bearing liposomes. Antimicrob. Agents Chemother. 32:966–970.

31. Tanford, C. 1980. The hydrophobic effect: formation of micelles and biological membranes, 2nd ed., p. 14–17. John Wiley & Sons, Inc., New York.

32. Wollensweber, H.-W., K. W. Broady, O. Luderitz, and E. T. Rietschel. 1982. The chemical structure of lipid A. Eur. J. Biochem. 124:191–198.