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Long-Chain Alkanoylcholines, a New Category of Soft Antimicrobial Agents That Are Enzymatically Degradable

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A new category of amphiphilic hydrolyzable quaternary ammonium compounds with rapid and high levels of antimicrobial activity was studied. The compounds, alkanoylcholines with hydrocarbon chains of 10 to 14 carbon atoms, are hydrolyzed by butyrylcholine esterase, which is present in human serum and mucosal membranes. The hydrolysis products are common components of human metabolism. Alkanoylcholines were tested and found to be active against gram-negative and gram-positive bacteria as well as yeasts. The microbicidal activities of the alkanoylcholines were comparable to the activities of the stable quaternary ammonium compounds of corresponding chain length and increased with an increasing number of carbon atoms. The compounds were also found to be hydrolyzed by enzymes present in certain microorganisms. The degradation was achieved after reaching the microbicidal effect.

Amphiphilic quaternary ammonium compounds are surface-active substances that are known for their membrane-disruptive effects and antimicrobial activities (10, 14). These compounds exhibit rapid activity against a broad range of microorganisms: gram-positive and gram-negative bacteria, fungi, and certain viruses. However, the high degree of affinity of these agents for biological membranes in general results in low selectivity, which can lead to damage to mammalian cells if exposure times are sufficiently long.

The side effects of this type of compound can be minimized by introducing a cleavable moiety into the molecule, such as an ester bond. This will give compounds that are more labile and that can be subject to hydrolytic degradation. The hydrolysis step should preferably lead not only to a nonactive compound but also to degradation products that are significantly less toxic than the original substance. Compounds based on this type of concept were originally developed by Bodor et al. (2) and were introduced as "soft" drugs. Soft drugs are defined as biologically active, therapeutically useful compounds characterized by a controllable degradation to nontoxic moieties after they achieve their therapeutic effect. This concept can be applied to limit the toxicities of antimicrobial agents, and the first soft drug to be introduced was actually an antimicrobial agent (2).

The work in our laboratory relating to soft antimicrobial agents has been centered around cationic amphiphilic compounds which degrade into natural products of human metabolism. In line with this work, we have earlier introduced long-chain alkyl betaine esters as soft antimicrobial agents (6, 12). These compounds were shown to have good microbicidal activity and are degraded to betaine and long-chain alcohols. The rate of hydrolysis of the compounds is pH dependent and was found to be highly influenced by the amphiphilic nature of the substances (22, 23).

In order to expand the field of soft antimicrobial agents to include enzymatically degradable compounds, we initiated studies with a series of alkanoylcholines with various hydrocarbon chain lengths (Fig. 1) (4). Our interest in these compounds was due to their potential degradation by hydrolytic enzymes. The hydrolysis products of long-chain alkanoylcholines, choline and fatty acids, are common human metabolites, and their metabolic pathways have been well investigated (13, 18).

Butyrylcholine esterase (BChE; EC 3.1.1.8), which is found in mammalian serum and mucosal membranes, is known to catalyze the hydrolysis of short-chain alkanoylcholines (19). Early studies of BChE indicated that butyrylcholine is the substrate with optimal activity and that long-chain alkanoylcholines are not hydrolyzed by the enzyme (9). We found, however, that the long-chain alkanoylcholines are hydrolyzed in vitro but that the reaction does not follow Michaelis-Menten kinetics and shows deviation from linearity caused by inhibition of the reaction at high substrate concentrations (4). We showed that the inhibition could be a result of conformational changes of the protein due to the amphiphilic nature of the substrates. Furthermore, the formation of mixed micelles between the substrate and the fatty acids formed during the hydrolysis reaction will lower the monomer concentration of the substrate, which could also partially explain the inhibition.

The in vitro hydrolysis studies were complemented with an investigation on the hydrolysis of alkanoylcholines in a biological environment, the rat intestine (5). These studies showed that alkanoylcholines are readily hydrolyzed in vivo and that the rate of hydrolysis decreases with increasing hydrocarbon chain length. Furthermore, the hydrolysis follows the occurrence of BChE through the rat intestinal tract (11, 20), indicating that BChE is responsible for the degradation of the amphiphilic alkanoylcholines.

The stabilities of the alkanoylcholines could also be influenced by hydrolytic enzymes of microbial origin, but the presence of BChE in microorganisms has not been reported previously.

This report describes the microbicidal activities of long-chain alkanoylcholines against a range of bacteria and yeasts as well as the hydrolytic effects on the substances of enzymes present in the microorganisms.

MATERIALS AND METHODS

Compounds investigated. The alkanoylcholine bromides were prepared as follows. Fatty acid chlorides (C_{10} to C_{14}) were dissolved in dry diethyl ether, and

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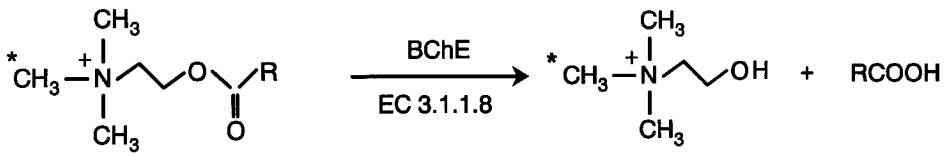

C10: R = C_9H_{19}

C12: R = $C_{11}H_{23}$

C14: R = $C_{13}H_{27}$

FIG. 1. Enzymatic hydrolysis of long-chain alkanoylcholines catalyzed by BChE.

the solution was added dropwise under stirring to a solution of *N*,*N*-dimethylaminoethanol in dry diethyl ether. The precipitates, 2-(*N*,*N*-dimethylamino)ethyl ester hydrochlorides, were filtered off and recrystallized from warm ethyl acetate. The products were further used for the syntheses of both labeled and unlabeled alkanoylcholines.

The aminoester hydrochloride was dissolved in water, and a saturated solution of Na_2CO_3 was added to liberate the free base. The solution was then extracted twice with diethyl ether, and the organic phase was dried with $MgSO_4$. An appropriate amount of [^{3}H]methyl iodide dissolved in toluene was added, and the solution, in a thoroughly closed vial, was left for 2 h at room temperature. An excess of methyl bromide was then added, and the mixture was left overnight. The precipitate was filtered off and washed with diethyl ether. The radiochemical purity of the products was verified by high-pressure liquid chromatography (HPLC) with on-line beta-radiation counting detection by calculating the areas of the peaks obtained in the chromatograms (24).

For the syntheses of unlabeled alkanoylcholines, the extraction of free amino ester was performed with ethyl acetate. Only the excess of methyl bromide was added, and the mixture was kept at 37°C for 1 h and then at room temperature overnight.

A more detailed description of the synthesis can be found in reference 3.

Electrospray ionization mass spectrometric analysis of the compounds gave spectra containing only the M^+ molecular ion (258, 286, and 314 for C_{10}, C_{12}, and C_{14}, respectively). However, increasing the accelerating voltage led to collision-induced dissociation and a certain extent of fragmentation. For C_{10}, an increase in the voltage from 30 to 100 V gave a spectrum with the molecular ion (258 *m*/*z*) and major fragmentation ions at 199 $[(CH_3)_3NCH_2CH_2OC(CH_2)_6]^+$, 85 $[(CH_3)_3NCH_2CH_2]^+$, 71 $[(CH_3)_2NCH_2CH_2]^+$, 57 $[CH_3NCH_2CH_2]^+$, and 43 $[CH_3NCH_2]^+$.

Instrumentation and chromatography. The liquid scintillation counter used in the study was an LKB 1217 instrument (LKB Wallac, Turku, Finland). The radiochromatography system was composed of an LKB model 2150/2152 dual pump gradient delivery system, a 100-μl loop, a 150-by-4.6-mm (inner diameter) analytical column packed with 5-μm Kromasil C_{18} (Eka Nobel, Surte, Sweden), and a Radiomatic A-250 radioactivity monitor. The detector was run in the homogeneous mode and was equipped with a 2.5-ml liquid scintillation cell.

The scintillator fluid/mobile phase ratio was 4:1. The mobile phases consisted of acetonitrile-water mixtures adjusted to pH 3.0 with hydrochloric acid, with a flow rate of 1 ml/min.

The electrospray ionization mass spectrometer used for structure confirmation was a Trio 2000 instrument (Fison Instruments, Altrincham, United Kingdom). Samples were dissolved in acetonitrile-water (1:1) and were introduced into the ion source at a flow rate of 10 μl/min.

Microorganisms and culture conditions. The antimicrobial activity was tested on *Salmonella typhimurium* 395 MS (serogroup B, S form) and 395 MR10 (R mutant, chemotype Rd), which have been described in detail elsewhere (7), and on *Salmonella braenderup* CCUG 12644 (serogroup C1); *Salmonella infantis* CCUG 12615 (serogroup C1); *Escherichia coli* CCUG 11412 and CCUG 11320; *Streptococcus agalactiae* CCUG 4208, CCUG 4209, and CCUG 4210; *Staphylococcus saprophyticus* CCUG 18736; *Campylobacter jejuni* (*C. coli*) CCUG 11701 and CCUG 11702; and *Lactobacillus casei* CCUG 21451 (Culture Collection, University of Göteborg, Göteborg, Sweden). Six *Candida albicans* and four *Candida glabrata* isolates were obtained from recently cultured clinical specimens. All bacteria and yeasts were maintained on agar slants in the cold.

The solid medium used for *S. typhimurium*, *E. coli*, and *S. saprophyticus* (group I) was nutrient agar, for *S. agalactiae*, *L. casei*, and *C. jejuni* (*C. coli*) (group II) the solid medium was blood agar, and for *C. albicans* and *C. glabrata* (group III) the solid medium was Sabouraud agar. The liquid media were nutrient broth, brain heart infusion broth, and Sabouraud broth, respectively.

All microorganisms were grown in 20 ml of broth, groups I and III were grown on a rotary shaker at 37°C for 18 h, *S. agalactiae* and *L. casei* were grown in a CO_2 (4.2%) atmosphere at 37°C for 48 h, and *C. jejuni* (*C. coli*) was grown in a microaerophilic atmosphere (5% O_2, 10% CO_2, 85% N_2) at 42°C for 48 h.

Microbicidal activities of alkanoylcholines. The susceptibilities of the microorganisms to the alkanoylcholines were determined by two different techniques. In both techniques, 20 ml of the bacteria and yeast cultures were harvested by centrifugation and were washed once with phosphate-buffered saline solution. The sedimented bacteria were resuspended in 2 ml of 0.1 M phosphate buffer (pH 8.0). The yeast suspensions were diluted to obtain an optical density of 0.7 absorbance units at 610 nm, and 40 ml of the cell suspension was then centrifuged and resuspended in 2 ml of 0.1 M phosphate buffer (pH 8.0). The microorganisms were exposed to serial dilutions of the esters in 0.1 M phosphate buffer at pH 8 and 37°C for 10 min.

The first of the techniques used the spiral plater system (Spiral Systems, Cincinnati, Ohio). The samples (1 ml per vial) were diluted 100-fold with 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 salts and is recommended as a neutralizing diluent for the evaluation of disinfectants containing cationic surfactants (1). The suspensions and dilutions were then plated onto nutrient agar.

The other method used a microtiter plate technique for the rapid determination of minimum microbicidal concentrations. Aliquots of 5 μl were taken from each well (0.2 ml per well) and were directly transferred onto nutrient agar plates after the 10-min exposure. All agar plates were incubated for 1 to 3 days in the appropriate atmosphere and at the appropriate temperature for the microorganism tested.

Uptake and hydrolysis of alkanoylcholines. Undiluted washed cell suspensions of the organisms (100 μl) were added to 900 μl of preincubated 0.1 M phosphate buffer (pH 8.0) which contained different concentrations of radiolabeled tetradecanoylcholine. After mixing, the suspensions were incubated at 37°C for 10 min, after which the cells were pelleted by centrifugation at 12,500 rpm (Microfuge E; Beckman) for 5 min. The radioactivity of the supernatant was measured either after separation by HPLC with on-line detection or with a liquid scintillation counter.

RESULTS

Antimicrobial effects of alkanoylcholines as a function of chain length. To determine the influence of the chain length of alkanoylcholines on the bactericidal effect, we tested a series of esters with 10 to 14 carbon atoms in the alkyl chain, decanoylcholine (C_{10}), dodecanoylcholine (C_{12}), and tetradecanoylcholine (C_{14}), against *S. typhimurium* 395 MR10 and *S. infantis* 12615 using the spiral plater technique (Fig. 2 and 3). In 0.1 M sodium phosphate buffer at pH 8.0 and 37°C, C_{14} showed the highest and C_{10} showed the lowest level of activity. C_{12} was only slightly less effective than C_{14}. There was no significant difference in the antibacterial effects on the two *Salmonella* strains.

We tested the same series of alkanoylcholines against *C. albicans* and *C. glabrata*. The yeasts were exposed to different concentrations of the esters at 37°C. The killing effects of the alkanoylcholines against both *Candida* strains increased, as expected, with an increase in the chain length of the ester (Fig. 4 and 5). The effect of the stable quaternary ammonium com-

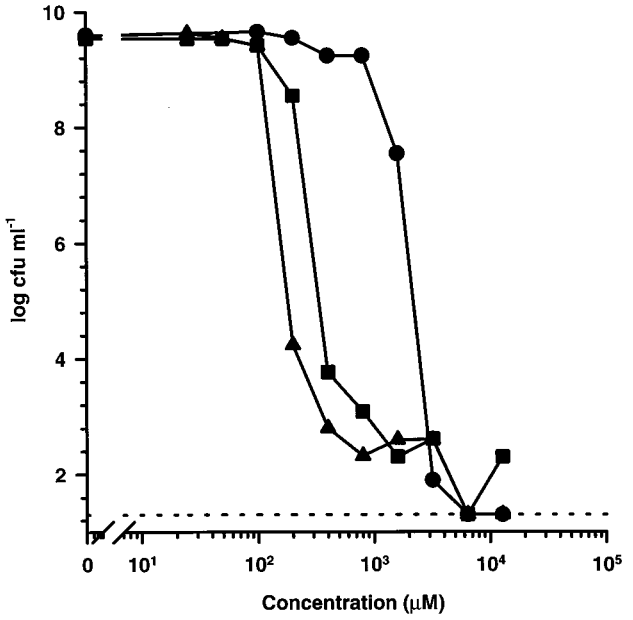

FIG. 2. Antimicrobial effects of long-chain alkanoylcholines against *S. typhimurium* 395 R10 in 0.1 M sodium phosphate buffer (pH 8) for 10 min at 37°C. ●, decanoylcholine (C_{10}); ■, dodecanoylcholine (C_{12}); ▲, tetradecanoylcholine (C_{14}).

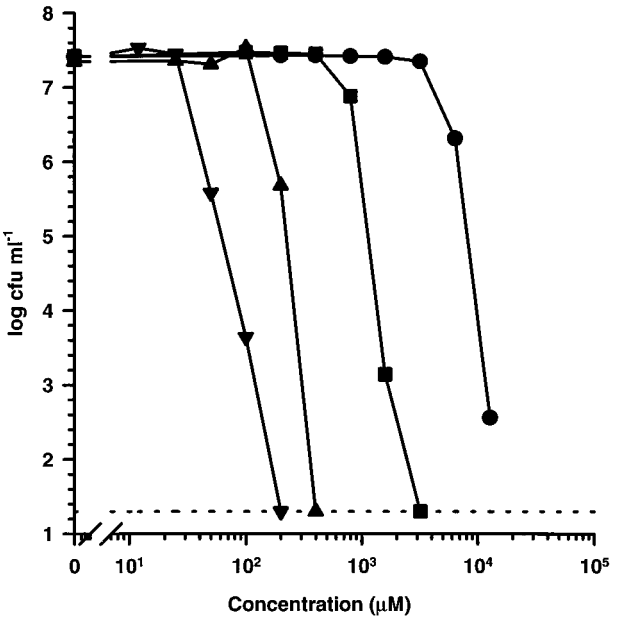

FIG. 4. Antimicrobial effects of long-chain alkanoylcholines and CTAB against *C. albicans* 796 in 0.1 M sodium phosphate buffer (pH 8) for 10 min at 37°C. ●, decanoylcholine (C_{10}); ■, dodecanoylcholine (C_{12}); ▲, tetradecanoylcholine (C_{14}); ▼, CTAB.

pound hexadecyltrimethylammonium bromide (CTAB) on *C. albicans* was tested as a comparison with those of the hydrolyzable cationic surfactants. CTAB was slightly more effective than C_{14}.

Determination of MBCs. The microtiter plate technique was used to determine MBCs and minimal fungicidal concentrations (MFCs) (Table 1). Low MBCs were found with C_{14} for both gram-positive and gram-negative bacteria. The only deviation was found with *S. saprophyticus*. C_{10} and C_{12} were only tested on *Salmonella* spp., and the activity of C_{12} was found to be in the same range as that of C_{14}.

MFCs were determined for *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. krusei*. The killing effect was similar for all species and increased with an increase in the alkyl chain length of the choline esters.

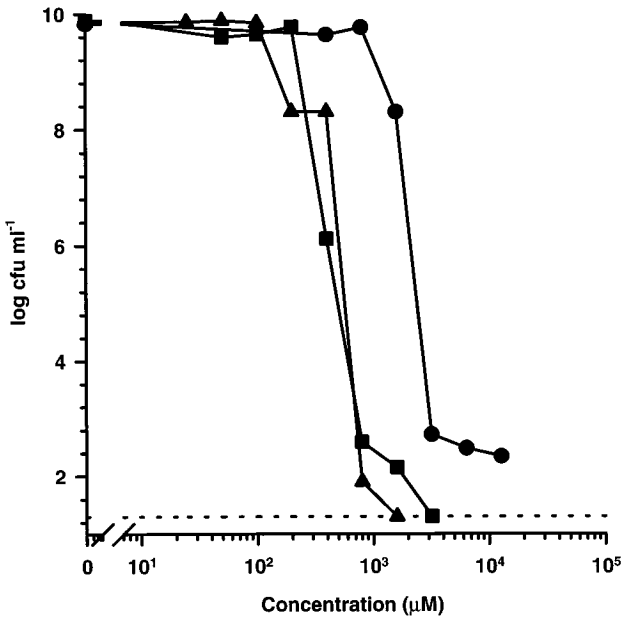

FIG. 3. Antimicrobial effects of long-chain alkanoylcholines against *S. infantis* in 0.1 M sodium phosphate buffer (pH 8) for 10 min at 37°C. ●, decanoylcholine (C_{10}); ■, dodecanoylcholine (C_{12}); ▲, tetradecanoylcholine (C_{14}).

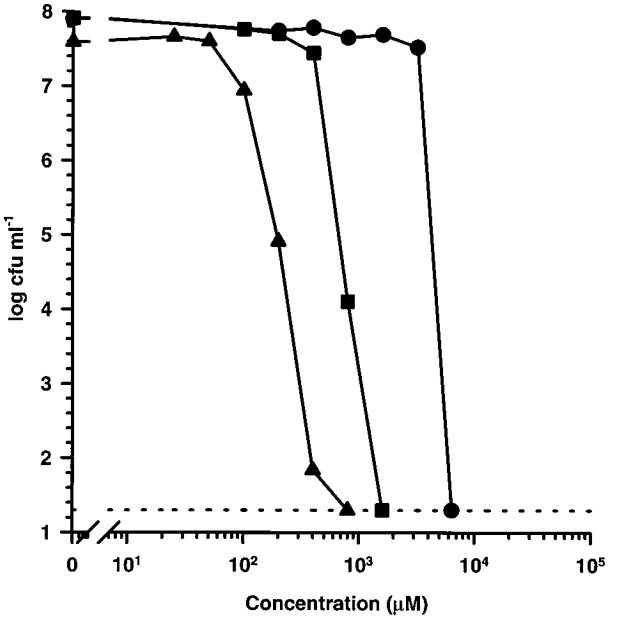

FIG. 5. Antimicrobial effects of long-chain alkanoylcholines against *C. glabrata* 868 in 0.1 M sodium phosphate buffer (pH 8) for 10 min at 37°C. ●, decanoylcholine (C_{10}); ■, dodecanoylcholine (C_{12}); ▲, tetradecanoylcholine (C_{14}).

TABLE 1. MBCs and MFCs of alkanoylcholines in 0.1 M phosphate buffer at 37°C for 10 min[a]

Organism	Alkanoylcholine concn (mM)		
	C_{10}	C_{12}	C_{14}
S. typhimurium 395 R10	3.2	0.4	0.2
S. infantis CCUG 12615	3.2	0.8	0.8
S. braenderup CCUG 12644	3.2	0.4	0.4
E. coli CCUG 11320			0.8
E. coli CCUG 11412			0.8
C. jejuni CCUG 11701			0.8
C. jejuni CCUG 11702			0.8
S. agalactiae CCUG 4208			0.8
S. agalactiae CCUG 4209			0.8
S. agalactiae CCUG 4210			0.4
S. saprophyticus CCUG 18736			3.2
L. casei CCUG 21451			0.8
L. casei CCUG 21452			0.8
C. albicans 796	>12.8	1.6	0.4
C. albicans 875			0.4
C. glabrata 868	6.4	1.6	0.4
C. glabrata 772			0.4
C. parapsilosis 983	>12.8	3.2	0.8
C. krusei 685	6.4	3.2	0.2

[a] Values calculated as 99.999% killing of the bacteria (2×10^9 to 7×10^9 ml^{-1}) and yeasts (2×10^7 to 7×10^7 ml^{-1}).

Degradation of tetradecanoylcholine in the presence of bacteria and yeasts. A survey of the capacity of different bacteria and yeasts to degrade C_{14} was performed by exposing the microorganisms to 0.4 mM [^{3}H]tetradecanoylcholine at 37°C for 10 min. The suspensions were then centrifuged, and the radioactivity in the supernatant was measured in a scintillation counter. Assuming that most of the nonhydrolyzed alkanoylcholine was bound to the cells, enhanced radioactivity in the supernatant would indicate that C_{14} was hydrolyzed and that choline was released. As can be seen in Table 2, the highest level of activity in the supernatant was found with the *Salmonella* species tested (67 to 79%) and with the *C. glabrata* isolates (69 to 100%). In contrast, *S. agalactiae* gave only 6% activity in the supernatant.

In order to ascertain the contribution of C_{14} to the radioactivity in the supernatant, the activities of the different components were determined after separation by HPLC. The differences observed between the strains (Table 3) were analogous to those recorded in Table 2. The most effective hydrolysis of C_{14} was found with *C. glabrata*, which gave 0.33 mM choline after 10 min from 0.4 mM ester (Table 3). The four *Salmonella* spp. showed hydrolysis that was almost as good as that of *C. glabrata*, e.g., 0.29 mM choline with *S. infantis.*

TABLE 2. Survey of capacity of bacteria and yeasts to hydrolyze alkanoylcholines by exposing the microorganisms to 0.4 mM [^{3}H]tetradecanoylcholine at 37°C for 10 min[a]

Organism	OD_{610}[b]	% Activity
S. typhimurium 395 R10	0.43	69
S. infantis CCUG 12615	0.50	79
S. braenderup CCUG 12644	0.50	67
E. coli CCUG 11320	0.34	19
E. coli CCUG 11412	0.30	19
C. jejuni CCUG 11701	0.32	14
C. jejuni CCUG 11702	0.62	22
S. agalactiae CCUG 4208	0.50	6
S. agalactiae CCUG 4209	0.52	6
S. agalactiae CCUG 4210	0.50	8
S. saprophyticus CCUG 18736	0.54	31
L. casei CCUG 21451	0.54	9
C. albicans 796	0.65	14
C. albicans 875	0.85	11
C. albicans 885	0.68	14
C. albicans 731	0.63	12
C. albicans 1381	0.64	9
C. albicans 11649	0.63	15
C. glabrata 1704	0.55	69
C. glabrata 772	0.26	100
C. glabrata 868	0.48	89
C. glabrata 882	0.26	98

[a] After centrifugation, the radioactivity in the supernatant was measured and was expressed as a percentage of the total radioactivity added.

[b] OD_{610}, optical density at 610 nm, measured after diluting bacteria 1/5 and yeasts 1/10 in buffer.

DISCUSSION

It has been established that cationic surfactants interact with the lipid bilayer structures of microbial cell membranes. The cationic polar head group of this class of compounds interacts with negative charges on the surface of the membrane, while the hydrocarbon chain is intercalated into the hydrophobic interior (14). The series of alkanoylcholines studied by us show differences in their antimicrobial effects, depending on the length of the hydrocarbon chain, since the choline part of the compounds is identical throughout the series. The highest degree of bactericidal and fungicidal activity was found for tetradecanoylcholine (C_{14}). The decyl derivative (C_{10}) lacked activity at concentrations of less than 2 mM for the bacteria and 5 mM for the yeasts under the experimental conditions used (Fig. 2 to 5). The differences in activity between C_{12} and C_{14} were largest against *C. glabrata* and *C. albicans*, less difference was observed against *S. typhimurium*, and the antimicrobial effects of both compounds were almost the same against *S. infantis*. These results are in good agreement with those of studies of the nonhydrolyzable amphiphilic ammonium compounds (8). In that study, optimal activity against bacterial strains and yeasts was found with chain lengths of 14 and 16, respectively. This can be explained by differences in the envelope and phospholipid compositions of the microorganisms, which affect interactions between the compounds studied and the cell membrane.

The results presented above are also in good agreement with those found with the long-chain alkyl betainates previously developed in our laboratory (12). Furthermore, the concentrations of both groups of hydrolyzable compounds necessary to give antimicrobial activity are comparable to those of the corresponding stable quaternary ammonium antimicrobial agents such as CTAB (Fig. 4) (12). As can be seen, CTAB shows a slightly greater antimicrobial effect than C_{14}. However, since the antimicrobial effect is dependent to a large extent on the number of carbon atoms in the hydrophobic chain, substances with similar chain lengths should be compared. The motivation for comparing C_{14} with CTAB (CTAB has 16 carbon atoms in the hydrophobic chain) is that for both the betaine and choline esters the critical micelle concentration is similar to that of a stable quarternary ammonium compound with two extra CH_2 groups, i.e., the critical micelle concentration of C_{14} is comparable to that of CTAB (4, 17). This would indicate that the ester function will be infused into the hydrophobic interior of the micelle. That this comparison of CTAB and C_{14} will also hold true in a system such as a bacterial membrane, however, cannot be taken for granted. Studies of transport kinetics in liposome lecithin membranes indicates that the flux of SO_4^{2-} across the membrane caused by the introduction of stable

TABLE 3. Concentration of free choline in the supernatant after hydrolysis of C_{14} by different microorganisms[a]

Organism	OD_{610}[b]	Concn (mM)	Choline concn (mM)	Ester concn (mM)
E. coli CCUG 11412	0.42	0.05	0.007	0.007
		0.1	0.02	0.02
		0.2	0.04	0.04
		0.4	0.04	0.07
		0.8	0	0.16
		1.6	0	0.38
		12.8	0	11.39
S. typhimurium 395 MS	0.42	0.1	0.07	0.001
		1.6	0.64	0.11
		12.8	0.90	10.62
S. typhimurium 395 R10	0.46	0.1	0.05	0.004
		0.4	0.27	0.01
		1.6	0.24	0.11
		12.8	0.38	11.14
S. infantis CCUG 12615	0.50	0.4	0.29	0.02
S. braenderup CCUG 12644	0.50	0.4	0.21	0.03
C. albicans 796	0.64	0.4	0.04	0.04
C. glabrata 868	0.48	0.4	0.33	0.004

[a] The results were obtained after exposure of different concentrations of [^{3}H]tetradecanoylcholine to bacteria and yeasts at 37°C for 10 min. The radioactivity was measured after separation by HPLC and on-line detection.

[b] OD_{610}, optical density at 610 nm, measured after diluting bacteria 1/5 and yeasts 1/10 in buffer.

quarternary ammonium compounds with 12 carbon atoms in the hydrophobic chain is similar to that for a betaine ester with the same number of carbon atoms (16). Furthermore, it is known that the stable quaternary ammonium compounds show maximum antimicrobial activity with chain lengths of 14 to 16 carbon atoms (8). We have earlier shown that for betaine esters the maximum activity is also found with chain lengths of 16 to 18 carbon atoms (12). Thus, for the alkanoylcholines a more appropriate comparison might have been CTAB and a C_{16} ester. However, for the alkanoylcholines, we have only tested chain lengths of up to 14 carbon atoms because of the negative effect on enzymatic hydrolysis by the more hydrophobic esters (4).

Tetradecanoylcholine, C_{14}, being the most effective ester, was chosen as the test substance against a representative selection of different bacteria and yeasts (Table 1). The MBC obtained showed that the antimicrobial effects of hydrolyzable quaternary ammonium compounds are excellent against gram-negative as well as gram-positive bacteria and are comparable to those of stable quaternary ammonium compounds. The MBC of C_{14} ranges between 0.4 and 0.8 mM. The low MBC (0.2 mM) for *S. typhimurium* 395 R10 may be due to the deep rough character of the outer membrane of the bacteria, making it both hydrophobic and accessible to surface-active agents (15). The deviation observed with *S. saprophyticus* (3.2 mM) is probably due to the fact that staphylococci grow in clusters, which impedes the accessibility of the antimicrobial agent to the individual cells and implies that in these cases there is more than one viable cell per CFU. Kinetic studies of biocide killing of cluster-forming bacteria show that plots of the natural logarithm of survivors versus time are convex, i.e., high survival rates with short exposure times (21). This multiple-hit phenomenon, together with the obstructed access of the antimicrobial agent to the individual cells, would be expected to give lower killing rates with the short exposure times used in the present study. However, if the exposure time is sufficiently long, the effect of C_{14} should be as good as that against the other microorganisms tested. The MFC of C_{14} against different *Candida* species is in the range of 0.2 to 0.8 mM. However, the antimicrobial effect is more chain length dependent with yeasts than with bacteria.

The primary idea of using alkanoylcholines as soft antimicrobial agents was that the compounds are readily degraded by BChE, which is present in mammalian tissues and serum (4). However, during the studies of the antimicrobial effect, it was found that some of the bacteria and yeasts investigated also catalyzed the hydrolysis of alkanoylcholines. Despite this, the killing effect of the compounds in 10-min exposures was not affected conspicuously. Thus, *C. glabrata* cells were killed by C_{14} as well as catalyzed the hydrolysis of the compound. Similar results were found with the different *Salmonella* spp., while the other microorganisms tested showed less or almost no hydrolysis capacity (Table 2).

The hydrolysis results in Table 3 show that the amount of choline released increases with the amount of added choline ester up to a plateau level. In the case of *E. coli*, however, no choline seems to be generated above a certain ester concentration. This could be due to a denaturation of the enzyme by excess ester. A similar effect was previously observed in vitro with BChE (4).

In conclusion, the long-chain alkanoylcholines show rapid antimicrobial activity against a wide range of microorganisms. At the same time, the esters are enzymatically hydrolyzable to nonactive components not only by BChE in mammalian systems, as shown earlier, but also by enzyme systems present in the microorganisms themselves. These substances are thought to have a good potential to replace the more toxic stable quaternary ammonium compounds.

ACKNOWLEDGMENT

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