Structure–Function Relationship of Acyl Amino Acid Surfactants: Surface Activity and Antimicrobial Properties

Jiding Xia,† Yongmei Xia,‡ and Ifendu A. Nnanna*,†

Department of Nutrition and Food Science, Room 3009 Science Hall, Wayne State University, Detroit, Michigan 48202, and Wuxi Institute of Light Industry, Wuxi, Jiangsu, People's Republic of China 214036

Amino acid surfactants (AAS), having the general structure α-amino-(*N*-acyl)-β-alkoxypropionate, were synthesized chemically. Surface activity and antimicrobial properties of the AAS were evaluated. Increases in acyl chain length (i.e., C_{10}–C_{14}) resulted in a linear reduction in surface tension (i.e., 43–36 $mN \cdot m^{-1}$), as well as dramatic decreases in critical micelle concentrations (cmc) (i.e., 17.9–0.43 mM). Strong correlations existed between the cmc of AAS and their minimal inhibitory concentrations (mic) against *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus niger*, and *Saccharomyces cerevisiae*. Sensitivity of the microorganisms to the various AAS followed the order *Staphylococcus aureus* > *A. niger* = *S. cerevisiae* > *E. coli* > *P. aeruginosa*. In comparison with methyl *p*-hydroxybenzoate, AAS (MN14) showed 2–8, 64, and 4–8 times the activity against Gram-negative bacteria, Gram-positive bacteria, and fungi, respectively. Surface adsorption and/or bifunctional binding to the cell membrane may account for AAS action on microorganisms.

Keywords: *Amino acid surfactants; surface activity; antimicrobial properties*

INTRODUCTION

Amino acid surfactants (AAS) have been the subject of many studies, primarily on their applications in pharmaceuticals, biomedical compounds, and household cleaning products (Moss and Lucas, 1977; Moriyama, 1978; McEntire and Gipson, 1978; Takehora, 1984; Iyer et al., 1985; Toda, 1989; Oe et al., 1992). Information concerning the subject of AAS is mostly found in the patent literature. Such information is usually centered around their molecular properties (functionality, shape, size, etc.) and not much on the molecular basis of their functions.

An important characteristic of surfactants is their amphiphilic structure. Compounds with such structure can be synthesized chemically for specific functions in food technology and agriculture. At present, we are unaware of any food application. Knowledge of AAS structure and their potential benefits as antimicrobial and/or surface-active agents may lead to fuller exploration of their application in foods. Such compounds would potentially function as preservatives and/or emulsifier adjuncts. Likewise, AAS may function as dispersing agents in many agricultural systems.

The potential use of amphiphilic molecules as antimicrobial agents has been of interest to many researchers. Kabara et al. (1975) and Kabara and Haitsma (1975) reported the activity and effectiveness of acyl aminimide derivatives as antimicrobial agents. Their compounds were active against both bacteria and yeast, activity being a function of chain length (Kabara and Haitsma, 1975). The acyl derivatives tested showed maximum activity at chain lengths of C_{14} and C_{16} (Kabara et al., 1975). Bistline et al. (1980) studied a series of fatty acid amides and anilides and found them to be highly active against Gram-positive but inactive against Gram-negative bacteria. Specifically, *N,N'*-diethyl amides of C_{12-14} fatty acid had minimal inhibitory concentrations (mic) values of 100 ppm or less (Bistline et al., 1980). Recently, Molinero et al. (1988) prepared protein-based amphoteric surfactants by condensation of N^{α}-lauroylarginine with amino acids from a collagen hydrolysate. The N^{α}-lauroylarginine dipeptides had surfactant and antimicrobial properties. The Molinero research team has also prepared neutral N^{α}-lauroylarginine dipeptides from pure amino acids (Infante et al., 1989). The surfactants were reported to be very water-soluble with good surface properties and antimicrobial activity (Infante et al., 1989). A review of most studies on AAS and peptide surfactants showed a lack of emphasis on structure–function relationships. Our present study was designed to evaluate the effect of varying hydrophobic groups or alkyl groups on the performance of AAS. This approach would provide fundamental information to enable selection of structures for further development and performance optimization. Also, such information would assist in producing tailor-made AAS for specific purposes.

The objective of this study was to develop new acyl amino acid surfactants with antimicrobial properties by varying the chain length of hydrophobic groups or alkyl groups. Relationships between the structure of AAS and their surface activity or (mic) were examined.

MATERIALS AND METHODS

Materials. All chemicals were of analytical grade or better. The microorganisms employed in this study consisted of Gram-negative organisms [*Escherichia coli* (EC) ATCC 10536 and *Pseudomonas aeruginosa* (PA) ATCC 27853], a Gram-positive organism [*Staphylococcus aureus* (SA) ATCC 6538P], and fungi [*Aspergillus niger* (AN) and *Saccharomyces cerevisiae* (SC)]. These microoganisms represent those frequently encountered in foods and in cosmetic clinical specimens. The EC, PA, and SA were obtained from the American Type Culture Collection, Rockville, MD. The fungi, AN and SC, were from the culture collection maintained at the Department of Fermentation, Wuxi Institute of Light Industry, Wuxi, China.

* Author to whom correspondence should be addressed [telephone (313) 577-2500; fax (313) 577-8616].
† Wayne State University.
‡ Wuxi Institute of Light Industry.

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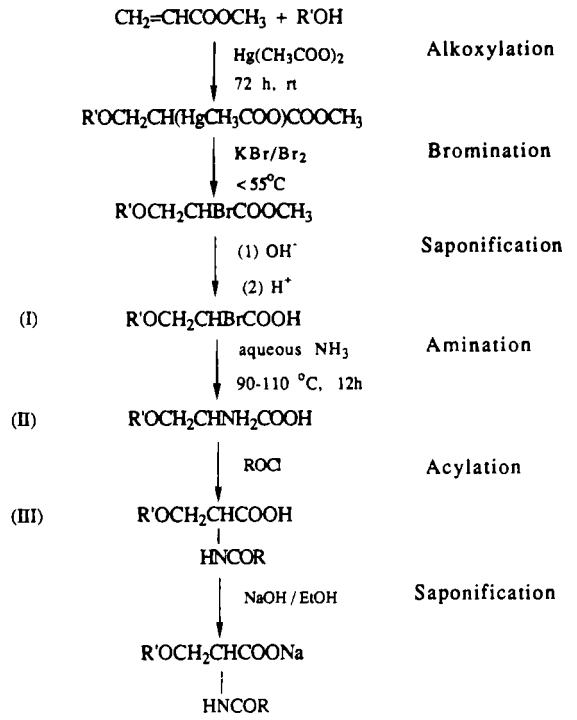


Figure 1. Synthetic scheme of the amino acid surfactants. (i) α-bromo-β-alkoxypropionic acid; (II) α-amino-β-alkoxypropionic acid; (III) α-amino-(*N*-acyl)-β-alkoxypropionic acid.

Methods. *General Structure of AAS.* The AAS studied are, by nomenclature, α-amino-(*N*-acyl)-β-alkoxypropionates and, in general, can be represented structurally as

$$\begin{array}{l} R'OCH_2CHCOONa \\ \quad\quad | \\ \quad HNCOR \end{array}$$

where R′ = H, CH_3, C_2H_5 and R = C_9H_{19}, $C_{11}H_{23}$, $C_{13}H_{27}$, $C_{15}H_{31}$.

A schematic representation of the synthetic route of AAS is presented in Figure 1. Below are brief descriptions of the procedures.

Synthesis of α-Bromo-β-alkoxypropionic Acid. Three hundred milliliters of propanoic acid methyl ester (CH_2=CH-$COOCH_3$) was added into a 1000 mL flask with three openings followed by the addition of appropriate amounts of mercury acetate and methyl alcohol. The mixture was stirred at room temperature for 72 h and then filtered to remove unreacted mercury acetate. Two hundred milliliters of 30% potassium bromide solution was added to the filtrate and allowed to stand at room temperature for 1 h. The mixture was extracted with chloroform, and the upper layer was washed with water. The washed liquor was dried by adding anhydrous magnesium sulfate followed by filtration to remove the residual $MgSO_4$. An appropriate volume of bromine was gradually added to the filtrate under violent stirring while the temperature was maintained at less than 55 °C. After removal of the precipitate (i.e., $HgBr_2$) by filtration, the crude filtrate was fractionally distilled under reduced pressure to obtain the ester bromide. After saponification and neutralization by NaOH, the ester bromide solution was extracted with ethyl ether followed by solvent removal by vacuum distillation. The intermediate product, α-bromo-β-methoxypropionic acid, was obtained (yield ~85%).

Synthesis of α-Amino-β-alkoxypropionic Acid. Aqueous NH_3 was combined with α-bromo-β-alkoxypropionic acid. The mixture was placed in a small autoclave equipped with a stirrer and allowed to react for 12–13 h at 90–110 °C with stirring. The reactants were concentrated to dryness. The solid product was recrystallized (3×) with 90% ethyl alcohol to obtain the product, α-amino-β-alkoxypropionic acid. The compound was identified by paper chromatography, and its structure was examined spectroscopically (IR and MS).

Table 1. Elemental Analysis of Acyl Amino Acid Surfactants

	alkyl groups		elemental composition (%)		
compd[a]	R′	R	C	H	N
MN10	CH_3	C_9H_{19}	61.09 (61.51)[b]	10.16 (9.95)	4.46 (5.12)
MN12	CH_3	$C_{11}H_{23}$	63.52 (63.38)	10.66 (10.37)	4.28 (4.65)
MN14	CH_3	$C_{13}H_{27}$	64.68 (65.62)	10.87 (10.71)	3.85 (4.25)
MN16	CH_3	$C_{15}H_{31}$	66.56 (67.19)	11.17 (10.99)	3.38 (3.92)
SN14	H	$C_{13}H_{27}$	63.31 (62.93)	10.49 (10.16)	3.59 (4.32)
EN14	C_2H_5	$C_{13}H_{27}$	66.09 (66.44)	11.06 (10.86)	3.69 (4.08)

[a] MN10, $CH_3OCH_2CH(NHCOC_9H_{19})COONa$; MN12, $CH_3OCH_2CH(NHCOC_{11}H_{23})COONa$; MN14, $CH_3OCH_2CH(NHCOC_{13}H_{27})COONa$; MN16, $CH_3OCH_2CH(NHCOC_{15}H_{31})COONa$; SN14, $HOCH_2CH(NHCOC_{13}H_{27})COONa$; EN14, $C_2H_5OCH_2CH(NHCOC_{13}H_{27})COONa$. [b] Values in parentheses are calculated values.

Synthesis of α-Amino-(N-acyl)-β-alkoxypropionic Acid and Na Salt Derivatives. α-Amino-β-alkoxypropionic acid was dissolved in acetone/water (1/1 v/v) solution followed by neutralization with equivalent solution of 2 N NaOH. The pH was brought to and maintained at ~9–13 by the addition of aqueous 2 N NaOH. Acyl chloride was added to the reaction vessel under vigorous stirring, and the mixture was acidified (pH ~1–2) with dilute HCl and filtered. The filtrate was washed with water and dried. The dried matter was rinsed with ethyl ether and recrystallized with solvent. Finally, white needle crystals were obtained (yield ~45%). The product, α-amino-(*N*-acyl)-β-alkoxypropionic acid [henceforth, acyl amino acid surfactant (AAS)], was identified and confirmed by IR and NMR. Elemental analyses were performed on all of the AAS; the data are shown in Table 1.

To obtain sodium salt derivatives, AAS was reacted with alcoholic NaOH solution at room temperature for 1 h. After solvent evaporation, the solid product was recrystallized to obtain a white powder (yield ~87%), α-amino-(*N*-acyl)-β-alkoxypropionate. Using different fatty acids, the above procedures allowed the preparation of decyl, dodecyl, tetradecyl, and hexadecyl acyl derivatives.

Determination of Surface Activity of AAS. The surface tension (γ) of AAS was determined by Wilhemy plate method (Hiemenz, 1977) using a Shimadzu ST-1 tensiometer in preequilibrated aqueous solutions (0.1%; 25 °C). In our testing, a standard glass plate with a 5 cm perimeter was utilized. The water used for preparing surfactant solution was first deionized and treated with alkaline permanganate and then distilled twice using a 50 cm condenser. The specific conductivity determined each day was about 1.1×10^{-6} mho cm^{-1}. Ten minute intervals were allowed for equilibration between measurements. Critical micelle concentrations (cmc) were obtained from the break-point of γ–log C curves (i.e., the minimum concentration of a surfactant solution at which the molecules of surfactant are transformed from unassociated molecules to micelles). In general, maximum surface reduction occurs at the cmc. Krafft point (°C) was determined using a 1% solution. Krafft point is the temperature at which there is a sudden change from turbidity to transparency during gentle heating of a 1% solution of ionic surfactant. It is a useful property of ionic surfactants, and it also represents the temperature at which their solubility is equal to their cmc. All analyses were performed in triplicate.

Antimicrobial Properties. To examine the antimicrobial properties of AAS, three bacterial species (*E. coli*, *P. aeruginosa*, and *S. aureus*) and two fungi (*A. niger* and *S. cerevisiae*) were tested in nutrient broth/agar. Methyl *p*-hydroxybenzoate, a common antimicrobial agent, was used as standard. Because nonionic surfactants have been found to abolish the antimicrobial effect of methyl *p*-hydroxybenzoate (unpublished data), it was of interest in this study to test for such possibilities with AAS. To achieve this, a nonionic surfactant, dodecyl octaoxyethylene glycol monoether ($C_{12}E_8$), was incorporated into MN14 at a 1:20 ratio ($C_{12}E_8$/MN14 w/w) and the antimicrobial activity of the mixture was tested as well.

Bacterial cultures, *E. coli*, *P. aeruginosa*, and *S. aureus*, were grown (24 h, 37 °C) in a peptone-enriched nutrient broth (composition: beef extract 0.3%, peptone 0.5%, and NaCl 0.5%). Stock cultures of the fungi, *A. niger* and *S. cerevisiae*,

Table 2. Surface Properties of Acyl Amino Acid Surfactants in Aqueous Solution

compd[a]	surface tension (γ)[b] ($mN \cdot m^{-1}$)	cmc (mM)	Krafft point (°C)
MN10	43.0 ± 4.3[c]	17.9 ± 2.5[c]	6 ± 0.3[c]
MN12	40.3 ± 3.7	5.19 ± 0.3	22 ± 1.3
MN14	36.6 ± 4.2	1.49 ± 0.1	31 ± 0.9
MN16	36.0 ± 2.8	0.43 ± 0.0	42 ± 1.1
SN14	47.2 ± 3.1	4.17 ± 0.1	17 ± 0.5
EN14	36.0 ± 1.9	1.20 ± 0.1	21 ± 1.3

[a] Chemical formulas of compounds are presented in footnote *a* of Table 1. [b] Surface tension was determined at 0.1% concentration and 25 °C. [c] Values represent mean ±SD of triplicate determination.

were maintained and preserved in glucose–nutrient broth (composition: glucose 0.1%, peptone 1%, NaCl 0.5%).

Minimal inhibitory concentration (mic) was determined by dilution test (Washington, 1985) using methyl *p*-hydroxybenzoate as standard. Serial dilutions of the AAS were made in nutrient broth to obtain surfactant concentrations of 3200, 1600, 800, 400, 200, 100, 50, 25, 12.5, and 0.0 μg/mL. The serial dilutions (pH 7.0–7.4) were inoculated with 0.1 mL of the test organisms and incubated at 37 °C for 48 h. After 48 h of incubation, the mic of each compound against each organism was determined by comparing bacterial growth in the presence of AAS with the control (i.e., 0.0 μg/mL AAS). The mic represents the lowest concentration (micrograms per milliliter) of AAS at which complete inhibition of the test organism occurred (Molinero et al., 1988).

To test the effect of AAS on fungi, a series of concentrations (i.e., 0–3200 μg/mL, as above) of AAS in sterile glucose–nutrient agar (composition: same as the broth with additional 2.0% agar) were prepared. The pH of the medium was ~7. A suspension of the inoculum was made in saline solution (0.1% NaCl), and the turbidity of the suspension was adjusted against barium sulfate standard. A 0.1 mL suspension of AN or SC was placed on the agar surface of the plates containing the dilutions of AAS. The control (i.e., 0.0 μg/mL AAS) and inoculated plates were incubated at 35 ± 1 °C for 5 days, after which the growth was examined visually. All testing was conducted in duplicate.

RESULTS AND DISCUSSION

Effect of Alkyl Chain Length on Surface Activity of Sodium Salts of AAS. Two important parameters frequently used to assess the efficacy of surfactants (short for surface-active agents) are their critical micelle concentrations (cmc) and their ability to reduce surface tension. The extent of surface tension reduction by a surfactant is a function of its adsorption property: the more the adsorption, the closer the packing of surfactant molecules at the interface. On the other hand, cmc is a measure of the tendency to form micelles, a phenomenon that is fundamental in the use of surfactants to solubilize water-insoluble material.

Table 2 presents the surface tension (γ), cmc, and Krafft points of AAS. As would be expected, Krafft points of AAS solutions increased with increase in acyl chain length. Increases in acyl chain length (i.e., from C_{10} to C_{14}) resulted in a linear reduction in surface tension (i.e., 43–36 $mN \cdot m^{-1}$). Dramatic decreases (i.e., from 17.9 to 0.43 mM) occurred in cmc as the acyl chain length increased from C_{10} to C_{14}. This observation is consistent with the trend reported by Rosen (1989) that as the number of carbon atoms in the hydrophobic group increases, a corresponding decrease in cmc is likely. The low cmc values of M14 and M16 suggest that they would be good solubilizers. In this study, strong linear relationship (R^2 = 0.959; Figure 2) existed between the chain length of the acyl group and log cmc. This relationship confirms the expected behavior of a homologous series of surfactants. That is, for a homologous series of surfactants, an increase in the length of the hydrocarbon chain as the series is ascended results in increased surface activity (Attwood and Florence, 1983). The relationship between hydrocarbon chain length and surface activity is expressed by Traube's rule, which states that "in dilute aqueous solutions of surfactants belonging to any one homologous series, the molar concentrations required to produce equal lowering of the surface tension of water decrease three-fold for each additional CH_2 group in the hydrocarbon chain of the solute" (Attwood and Florence, 1983). We found that a simple case such as the removal of CH_2 from the MN14 structure or the addition of CH_2 to it altered its surface activity remarkably.

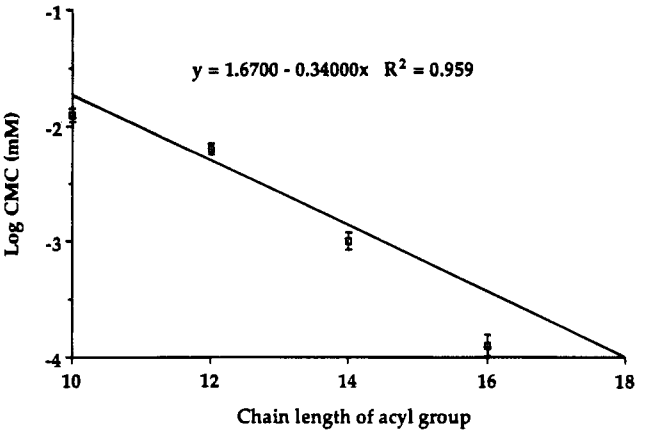


Figure 2. Relationship of acyl group chain length of AAS to critical micelle concentrations (cmc). Data points represent mean ± SD of triplicate measurements.

Removal of a CH_2 group from the MN14 acyl chain (MN14 → SN14) resulted in a 3-fold increase in cmc as well as dramatic changes in surface tension and Krafft point (Table 2). The SN14 derivative showed a decreased surface activity, as evident from its cmc and surface tension values compared to those of MN14 (SN14 = 47.2 $mN \cdot m^{-1}$ vs MN14 = 36.6 $mN \cdot m^{-1}$; Table 2). However, the addition of a CH_2 group to the MN14 structure to obtain EN14 caused little change in cmc (Table 2), but the Krafft point was reduced by one-third. This implies that EN14 would be more soluble in water at a lower temperature than MN14.

In general, changes in hydrophobic (acyl) chain length caused a greater effect on AAS surface properties than was seen with changes in the β-alkoxy group (alkyl group).

Antimicrobial Properties of AAS. The antimicrobial properties of AAS were tested against two Gram-negative and one Gram-positive bacteria, one mold, and one yeast. As can be seen from the mic values in Table 3, AAS exhibited broader antimicrobial effects than methyl *p*-hydroxybenzoate. The degree of effectiveness of the various AAS was dependent on their acyl chain length and on the microbial species used (Table 3). The AAS exhibited inhibitory activity against both Gram-positive and Gram-negative bacteria. However, the AAS showed lower activity against *P. aeruginosa*, as judged by the mic values (Table 3). For the most part, MN14, EN14, and MN14 + $C_{12}E_8$ showed the highest antimicrobial activity against the bacteria and fungi. Although the antibacterial activity of MN16 (C_{16}) was relatively minimal, its antifungal activity was comparable to those of the C_{14} derivatives, MN14 and EN14 (Table 3). The differences in antibacterial activity between C_{14} and C_{16} derivatives may be attributed to a

Table 3. Minimal Inhibitory Concentration (mic) of Amino Acid Surfactants for Different Microorganisms

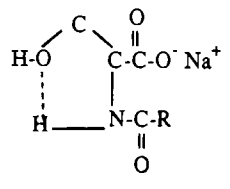
	mic (mg/mL) for microorganisms				
	Gram (+)	Gram (−)		fungi	
compd[a]	SA[b]	EC	PA	AN	SC
MParaben[b]	3200	>3200	>3200	800	800
MN10	800	200	3200	1600	1600
MN12	400	400	3200	800	800
MN14	50	400	1600	100	200
MN16	800	800	>800	200	200
SN14	>800	>800	>800	1600	1600
EN14	50	200	1600	200	200
MN14 + $C_{12}E_8$[c]	50	400	800	50	400

[a] Chemical formulas of compounds are presented in footnote *a* of Table 1. [b] SA, *S. aureus*; EC, *E. coli*; PA, *P. aeruginosa*; AN, *A. niger*; SA, *S. cerevisiae*. [c] Methyl *p*-hydroxybenzoate. [d] Mixture of MN14 and dodecyl octaoxyethylene monoether [$C_{12}H_{23}O(CH_2CH_2O)_8H$].

restricted penetration of C_{16} structure through the bacterial cell wall (Kabara and Haitsma, 1975).

To assess the effect of β-alkoxy groups (i.e., H, CH_3, and C_2H_5; see Table 3) on antimicrobial activity, compounds SN14, MN14, and EN14, respectively, were examined. SN14 was found to be less effective than the MN14 and EN14 counterparts (Table 3). It is noteworthy that a substitution of CH_3 by a hydrogen atom could change the antimicrobial activity dramatically. Such a simple substitution increased the mic values greatly (MN14 vs SN14; Table 3).

To understand the molecular basis of the differences in antimicrobial performance of MN14 , EN14, and SN14, some relevant insights may be formulated based on theoretical viewpoints and our experimental observation. MN14 and EN14 contained ether linkages in their structures, whereas SN14 had a hydroxyl group, which are likely to influence their dispersibility or interaction with media components. From this viewpoint, we suspect that the substitution of CH_3 by a hydrogen atom in the structure of MN14 to form SN14 resulted in an increased tendency of the latter to be involved in intramolecular hydrogen bonding. On the basis of this reasoning, two forms of intramolecular H-bonds are possible with SN14:



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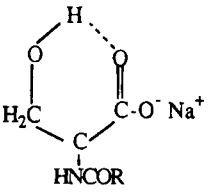

(II)

However, because of steric consideration, structure II is likely to be more stable. Such H-bonding may be expected to decrease the solubility of SN14 in culture, increase the steric retarding effect, or decrease the polarity of the hydrophilic group, effects which, singly or in combination, may decrease the binding between SN14 and the microbial surface. This view was based on the observation that the culture media instantaneously became turbid upon the incorporation of SN14, especially in culture media containing inorganic salts and proteins. The turbidity may be due to a decrease in the solubility of SN14 in culture. This may reduce the effective concentration of SN14 and could explain why it was less effective as an antimicrobial agent compared to MN14 and EN14 (Table 3).

From the data reported in Table 3, the antimicrobial efficacy of the various AAS against the microorganisms studied seemed to be in the following order: *S. aureus* > *A. niger* = *S. cerevisiae* > *E. coli* > *P. aeruginosa*. In comparison with methyl *p*-hydroxybenzoate, AAS showed ~2.6–60-fold activity against the Gram-negative and Gram-positive bacteria and fungi. It is noteworthy that the blend of a nonionic surfactant ($C_{12}E_8$) with MN14 caused no loss of activity, a principal effect nonionic surfactant has on methyl *p*-hydroxybenzoate. The incorporation of nonionic surfactant into MN14 not only facilitated more uniform dispersions of water-insoluble compounds (data not shown) but endowed it with a positive synergistic antibacterial effect against the most insensitive (in this study) bacteria, *P. aeruginosa* (Table 3). Although all of the surfactants evaluated in this study showed antimicrobial activity to some degree, Kabara and Haitsma (1975) cautioned that compounds that are surface active do not automatically qualify as effective bactericidal agents.

It is clear from this study that the structure of AAS affected their antimicrobial activity. Although several mechanisms may be conjectured for the antimicrobial actions of the AAS, activity may be due, in part, to their ability to adsorb on and/or penetrate the microbial cell wall and, eventually, the cell membrane. Bacteria interact avidly with cationic surfactants, converting their negatively charged surfaces to positively charged (Attwood and Florence, 1983). This is so because the bacterial cell wall is characteristically polyanionic (Infante et al., 1985). For this reason, cationic surfactants are predisposed to interact electrostatically with the cell wall surface. Indeed, Infante et al. (1985) found that only the surfactant derivatives with cationic charge were able to inhibit the growth of microorganisms. To explain their findings, Infante and co-workers proposed that the mechanism of action of the cationic surfactants may be due to the following sequence of events: adsorption of the surfactants to the bacterial cell wall followed by interaction of the polyanionic components of the cell surface with cationic surfactants. These events, consequently, weaken the cell wall integrity, allowing the surfactant to penetrate the cell membrane and prevent microbial growth (Franklin and Snow, 1981). The AAS investigated in this study were fundamentally anionic. They may likely behave as amphoterics depending on pH. Although no attempt was made by us to study the antimicrobial mechanism of AAS in the pH range 7.0–7.4, AAS may be expected to behave amphoterically. The relevance of this possibility to the antimicrobial activity remains to be investigated. Also to be investigated is the role of hydrophobic interaction between AAS and the microbial cell wall.

In consideration of the structural diversity between bacteria and fungi, it should be obvious that no one mechanism explains the antimicrobial activity of the AAS studied here. Although at the membrane level the function and basic structure of the bacteria and fungi plasma membranes are very similar, diversity and distinct differences exist between the cell wall compositions of bacteria and fungi (Brock et al., 1984). Both Gram-positive and Gram-negative bacteria have peptidoglycan cell walls, but Gram-positive bacteria have thicker cell walls (i.e., much higher peptidoglycan content) and lack lipopolysaccharide and other components of Gram-negative bacteria (Brock et al., 1984). Some surfactants can increase the permeability of the bacterial cell wall (Attwood and Florence, 1983) by virtue of the cell wall composition. The principal structural component of the cell wall of fungi is the polysaccharide chitin, a polymer of *N*-acetylglucosamine (NAG) units (Brock et al., 1984). Specifically, yeast cell

Table 4. Correlation between log mic for Microorganisms and log cmc of AAS

microoganism	regression[a]	correl coeff (R^2)
S. aureus	$Y = -1.4742 + 0.3611X$	0.080
E. coli	$Y = -11.843 - 3.1745X$	0.878
P. aeruginosa	$Y = 1.8777 + 1.9712X$	0.876
A. niger	$Y = 0.6358 + 1.0917X$	0.788
S. cerevisiae	$Y = 1.4676 + 1.4123X$	0.940

[a] Y = log mic; X = log cmc.

walls contain the polysaccharides glucan and mannan. It is reasonable to assume that the accessibility of the surfactants to the microbial interior would be controlled by cell wall makeup. Therefore, the mode of action of the AAS should be expected to differ for the bacterial species and fungi.

It is our opinion that the high antimicrobial activities of the "medium chain derivatives" MN14 and EN14 were due to their solubility in test medium, which facilitates dispersibility and increases effective surface area. This in turn could facilitate bacterial surface adsorption by the AAS and consequently membrane penetration. The shorter chain MN10 and MN12 exhibited more Gram-negative effect than did the longer chain (C_{16}) derivative. Presumably, the shorter chain derivatives can adsorb on and bind with the hydrophilic site of proteins and phospholipids, while the longer chain derivatives have restricted access to penetrate the lipopolysaccharide layer of Gram-negative bacteria.

Relationship between cmc and mic. Micelle formation is the basis of surface activity. It is reasonable to expect a relationship between cmc and antimicrobial activity. To verify this possibility, linear regression was constructed between log cmc of AAS and the corresponding log mic for each tested organism. Table 4 presents the correlations of the regression analyses. Correlation coefficients were 0.878, 0.876, 0.788, and 0.940, respectively, for *E. coli*, *P. aeruginosa*, *A. niger*, and *S. cerevisiae*, implying that the cmc of AAS played a major role in their antimicrobial activity. No relationship (correlation coefficient = 0.08) existed between the mic of AAS for *S. aureus* and the corresponding cmc. It is interesting to note that most of the mic values of AAS (Table 3) are lower than the corresponding cmc values (Table 2). This suggests that the AAS exhibited antimicrobial activity when in monomeric form and not in micelle form (i.e., aggregates).

Conclusion. In general, changes in hydrophobic (acyl) chain length caused greater effect on AAS surface properties than was seen with changes in the β-alkoxy group (alkyl group). As acyl chain length was increased (i.e., C_{10}–C_{14}) corresponding reduction in surface tension and critical micelle concentrations (cmc) occurred. AAS inhibited some Gram-positive and Gram-negative bacteria and generally showed broader antimicrobial effects than methyl *p*-hydroxybenzoate. Strong correlations existed between cmc of AAS and their mic against *E. coli*, *P. aeruginosa*, *A. niger*, and *S. cerevisiae*, implying that the cmc of AAS played a major role in their antimicrobial activity. Incorporation of nonionic surfactant ($C_{12}E_8$) into MN14 produced a positive synergistic antibacterial effect against *P. aeruginosa*. However, antimicrobial activities of AAS were only examined at about neutral pH (i.e. 7.0–7.4).

At this preliminary stage in our work, no attempt was made to evaluate the following: antimicrobial mechanism(s), toxicity, or food application potentials of AAS. It is too soon to foresee food applications. Nevertheless, the use of AAS in food flavor development is worth investigating. Also, further work is needed to explore the potential application of AAS in nonfood areas such as in cosmetics or detergents as mild preservatives.

ACKNOWLEDGMENT

Microorganisms were provided by the Department of Food Science, Wuxi Institute of Light Industry.

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Received for review June 9, 1994. Revised manuscript received December 12, 1994. Accepted January 25, 1995.[⊗]

JF940310Z

[⊗] Abstract published in *Advance ACS Abstracts*, March 1, 1995. Please note correction of the abstract. The fourth sentence should read "Strong correlations existed between the cmc of AAS and their minimal inhibitory concentrations (mic) against *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus niger*, and *Saccharomyces cerevisiae*".