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Surface-Bonded Antimicrobial Activity of an Organosilicon Quaternary Ammonium Chloride

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The hydrolysis product of 3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride exhibited antimicrobial activity against a broad range of microorganisms while chemically bonded to a variety of surfaces. The chemical was not removed from surfaces by repeated washing with water, and its antimicrobial activity could not be attributed to a slow release of the chemical, but rather to the surface-bonded chemical.

For over a decade alkoxysilanes have been utilized by a number of industries as coupling agents to reinforce or impart desirable properties to a variety of materials. Plueddemann (9) has reviewed their use for such purposes. More recently Weetall and co-workers (13, 14) have described their use as coupling agents for insolubilizing enzymes on inert surfaces. A number of enzymes were found to remain biologically active when bonded to inorganic surfaces by alkoxysilanes. Their activity was shown to persist after repeated washing procedures. The immobilization of enzymes altered their activity as evidenced by changes in stability and *p*H optima. Hough and Lyons (5) have further advanced this technology with the successful demonstration of the coupling of enzymes to yeast cells. While this manuscript was in preparation, Venter et al. (11) reported the successful covalent coupling of catecholamines to glass beads resulting in biologically active particles.

3-(Trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride (Si-QAC) is representative of a group of alkoxysilanes which have been under investigation in our laboratory over the past 3 years. This compound possesses antimicrobial activity when tested by a conventional serial tube dilution method. Similar alkoxysilanes in aqueous systems have been shown by Johannson et al. (6) to release methanol and form chemical covalent bonds with reactive surfaces (Fig. 1). The present study was conducted to determine whether the compound possessed antimicrobial activity when chemically bonded to a surface. This report was presented in part at the Annual Meeting of the American Society for Microbiology, Philadelphia, Pa., 23-28 April 1972.

MATERIALS AND METHODS

Chemicals. Si-QAC and ^{14}C-Si-QAC (labeled in the alkyl portion of the molecule) were prepared by J. R. Malek (Dow Corning Corp.) as methanolic solutions containing 50% solids (w/v) by a modification of the method of Speier et al. (10). Benzalkonium chlorides used in this work were alkylbenzyldimethyl ammonium chlorides (approximately 40% C_{12}; 50% C_{14}; 10% C_{16}) commercially available from Winthrop Laboratories, N.Y.

Organisms. Stock cultures of *Escherichia coli* B (ATCC 23226) and *Streptococcus faecalis* (ATCC 9790) were maintained on nutrient and Trypticase soy agar slants (Difco), respectively, at 4 C with monthly transfers. For preparation of inocula, *E. coli* B was transferred in nutrient broth for 3 consecutive days. *S. faecalis* was similarly transferred in Trypticase soy broth. The third 24-hr culture of each was harvested and washed in sterile saline by centrifugation at 15,000 rev/min for 15 min at 4 C in a Sorvall RC2-B centrifuge. Washed cell suspensions were resuspended in sterile saline to various optical densities (Coleman model 14 spectrophotometer) for aerosol inoculation of surfaces.

Mixed fungal spore suspensions of *Aspergillus niger* (ATCC 9642), *Aspergillus flavus* (ATCC 9643), *Aspergillus versicolor* (ATCC 11730), *Penicillium funiculosum* (ATCC 9644), and *Chaetomium globosum* (ATCC 6205) were prepared and applied as described in Military Standard-810B (8).

Test surfaces. Glass surfaces and cotton cloth were washed in detergent (Haemo-Sol) and rinsed exhaustively in tap water. Washed materials were then rinsed with 50% (v/v) isopropyl alcohol, followed by several distilled water washes before drying at 70 C for 30 min. Samples removed from the oven were allowed to stand at room temperature for 30 min under aseptic conditions prior to immersion in a bath of 0.1% Si-QAC or quaternary ammonium chloride (QAC) for 10 min. Chemically treated samples were again dried at 70 C for 30 min and allowed to remain

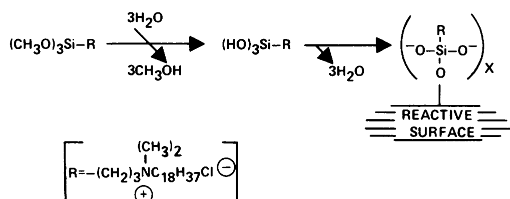

FIG. 1. *Hydrolysis and condensation of 3-(trimethoxysilyl)-propyldimethyl ammonium chloride with surfaces containing reactive functional groups.*

at room temperature in sterile petri dishes for 30 min before inoculation.

Radioactive cellulose acetate. This material was prepared by immersion of a 2-inch (ca. 5 cm) square of secondary cellulose acetate (four acetoxy groups per cellobiose unit) sheet, 1 mil (0.0254 mm) thick, without plasticizer or lubricant (Celanese Corp., Summit, N.J.), into a 0.1% solution of ^{14}C-Si-QAC in distilled water (specific activity 0.062 mCi/g), for 4 hr at 65 to 70 C. The square was then washed with two 20-ml portions of water. Further washing failed to remove radioactive material detectable by ^{14}C analysis of the sheet or of the wash water with a Packard Tri-Carb, model 3320 liquid scintillation counter. Samples of treated and untreated sheet (1 by 2 inches) were subjected together to aerosol contamination with *E. coli* B.

Bacterial test procedure. Treated surfaces plus untreated controls were subjected to aerosol inoculation in a polyvinyl chloride chamber designed for this purpose which was cylindrical and measured 47.5 inches (120.6 cm) in length by 11.8 inches (30 cm) in diameter. Test surfaces were placed on a wire support 6 inches (15.2 cm) from the bottom of the chamber, and the suspension of bacteria was aerosolized (average droplet size is less than 10 μm in diameter) onto the test surfaces by use of a pneumatic atomizer 1/4 J (Spray Systems Co., Wheaton, Ill.). Inoculated surfaces were held in sterile petri dishes at 25 C for 30 min before quantitation of viable organisms on the surface was accomplished by either wash recovery (1) or Rodac plate (2) techniques.

RESULTS

The antimicrobial activity of glass surfaces exposed to Si-QAC and QAC against *S. faecalis* is compared in Table 1. Recovery of 1,000 organisms from the control was used as a base line to calculate the reduction in the number of organisms caused by exposure of the glass surface to varying degrees, inoculated, and the reduction in surface contamination measured. Each sample was run in triplicate. On surfaces exposed to QAC, with no washing, 750 out of 1,000 organisms survived compared to two survivors on the Si-QAC-exposed surfaces. One 4-min wash permitted 100% survival of *S. faecalis* on the QAC-treated surface, indicating zero control of organisms. In contrast, after 50 washes, or 200 min, glass treated with Si-QAC effected a 95% decrease in surface contamination.

Cotton cloth identically treated (Fig. 2) was subjected to a mixed fungal spore suspension to demonstrate the relative antifungal activity of Si-QAC and QAC. Sample A is an untreated control. The zone of inhibition surrounding sample B, treated with QAC, demonstrates leaching of QAC. An identical sample, C, after

TABLE 1. *Survival of Streptococcus faecalis on glass surfaces*

No. of washes[a]	Viable organisms on surface[b]		
	No surface treatment	QAC surface treatment	Si-QAC surface treatment
0	1,000	750[c]	2
1	1,000	1,000	1
2	1,000	1,000	4
3	1,000	1,000	2
10	1,000	1,000	3
30	1,000	1,000	20
50	1,000	1,000	50

[a] Each wash consisted of 4 min under running tap water at 20 C.

[b] Standard error = ±10.

[c] Significantly different from the controls ($P < 0.05$).

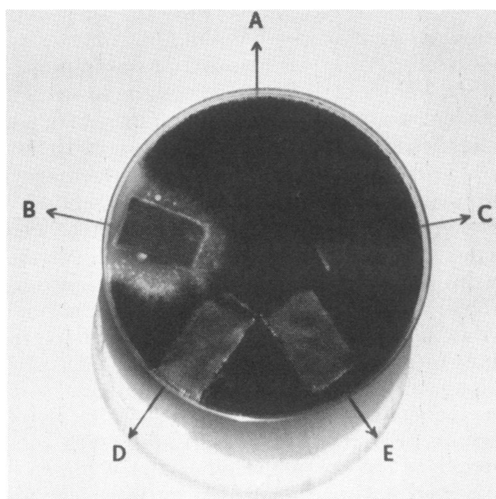

FIG. 2. *Antifungal protection of leached and nonleached cotton samples treated with: (A) untreated control; (B) QAC; (C) QAC plus 4-min water wash; (D) Si-QAC; (E) Si-QAC plus 4-min water wash.*

TABLE 2. *Durability of bonded ^{14}C-Si-QAC on cellulose acetate film to water washing and bacterial exposure*

Sample	Cellulose acetate treatment	*E. coli* B[a]	Wash[b]	Bacteria/ml	Disintegrations per min per ml (±S.E.)
Bacterial control	None	1,000	10 ml	980	27.5 (±2.52)
[14]C-Si-QAC control	^{14}C-Si-QAC (10 μg)	None	10 ml	None	27.0 (±2.57)
Test sample	^{14}C-Si-QAC (10.2 μg)	1,000	10 ml	73	26.8 (±2.57)

[a] Samples exposed to aerosol inoculation followed by incubation in sterile petri dish at 37 C for 30 min; S.E. ± 12.
[b] Wash consisted of shaking sample in closed container with 10 ml of sterile distilled water at 150 rev/min for 15 min.

one 4-min wash under running tap water was not protected from fungal growth, indicating the easy removal of QAC. Both samples treated with Si-QAC (D, unwashed and E, washed) were not attacked by fungi. Further, the lack of a zone of inhibition around either sample D or E suggests no loss of chemical to the environment surrounding the sample.

[14]C-Si-QAC-treated cellulose acetate sheet was used to gain further evidence of substantive antimicrobial activity of Si-QAC (Table 2). The bacterial control consisted of a 1-inch square of untreated cellulose acetate film, sprayed with *E. coli* B. The ^{14}C-control was the treated cellulose acetate without exposure to microorganisms, and the test sample was ^{14}C-treated cellulose acetate with *E. coli* B. After inoculation of the samples, all three were placed in sterile petri dishes at 37 C for 30 min. At the end of this time each film was placed in a vial containing 10 ml of sterile distilled water and shaken at 150 rev/min for 10 min, and samples of the water were taken for bacterial count and ^{14}C analysis.

Although the sample treated with Si-QAC effected a greater than 92% reduction in *E. coli* compared to the bacterial control; no loss of ^{14}C-labeled material from the cellulose acetate film due to either the wash procedure or exposure to microorganisms was detected.

To detect release of Si-QAC below the level of radioisotope analysis, a bioassay was used (Fig. 3). Tube A was a bacterial growth control. All tubes used in this experiment contained 10 ml of sterile broth. The broth was inoculated with 0.1 ml of a 24-hr culture of *E. coli* B and was incubated at 37 C for 24 hr on a rotary shaker at 150 rev/min, and plate counts were made. Tube B contained a 1 by 2 inch cellulose acetate sheet with approximately 10 μg of ^{14}C-Si-QAC bonded to the surface as measured by ^{14}C analysis. Tube C contained 10 μg of Si-QAC added to the broth or that amount which would be released from the cellulose acetate in tube B

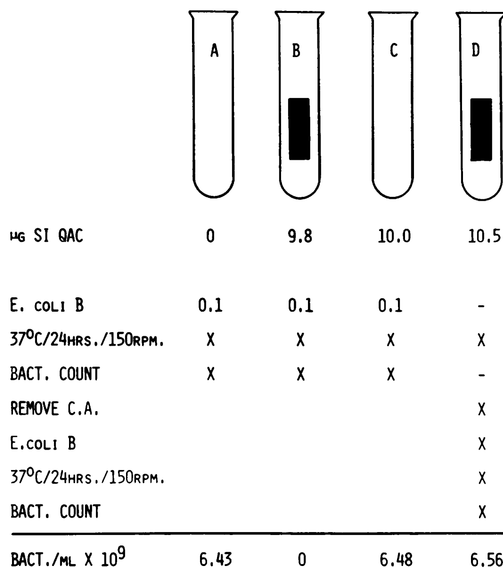

FIG. 3. *Bioassay determination of Si-QAC release from chemically treated cellulose acetate (CA) film samples.*

if 100% of the Si-QAC were leached into the broth. Both tubes B and C were inoculated, incubated, and counted in an identical manner to tube A. It can be argued that any activity due to slow release of material from the cellulose acetate film in tube B may not be chemically comparable to the direct addition of Si-QAC to the broth as in tube C. Tube D was thus included to control this variable, i.e., a treated cellulose acetate film identical to that used in tube B, but uninoculated for 24 hr and incubated under the same conditions. At the end of this time, the film was removed, and the remaining broth was inoculated, incubated, and counted. The broth exposed to the treated Si-QAC film for 24 hr was not antimicrobial. Thus, both the radioactive and bioassay data indicate that Si-QAC is an effective antimicrobial agent when firmly bonded to a surface,

and its activity is not dependent on slow release of the compound.

A number of substrates (Table 3) were found to exhibit durable antimicrobial activity when treated with Si-QAC, against a spectrum of microorganisms (Table 4) of medical and economic importance.

DISCUSSION

Vol'f (12) reported that nitrophenylacetate, nitrophenylphosphate, and "certain other agents" formed covalent bonds with polyvinyl alcohol fibers. The resultant fibers acquired a relatively permanent antibacterial (staphylococci, intestinal bacillus) antifungal (candida, trichophyton, epidermophyton), and antiprotozoal (trichomonas) activity. Vol'f states, however, that to achieve this activity the bond between the fiber and the active agent must be weakened by heating the fiber in a moist state or by washing in detergents of an acid nature to allow transportation (diffusion) of these groups within the microbial cells. In a similar manner Davis (3) attempted to produce antimicrobial surfaces through the slow release of hexachlorophene from polyethylene plastic surfaces.

The evidence presented indicates that the organosilicon quaternary ammonium salt we have investigated does not have to enter the cell for bactericidal activity. Although the antimicrobial activity of quaternary ammonium salts was first extensively examined by Domagk (4) in 1935, the specific site of action of this class of compounds is still being investigated. Their activity has been attributed by various workers to membrane phenomena, i.e., membrane lysis, membrane enzyme inactivation, or interference with ion transport (7). By chemical analogy, our data would lend support to the thesis that the site of action may be the membrane or cell wall, but probably not intracellular organelles.

The biological activity of Si-QAC bonded to surfaces may offer a method of surface protection without addition of the chemical to the environment. The treatment of solutions (serum, water, etc.) by passage over Si-QAC-

TABLE 3. *Si-QAC-treated substrates exhibiting antimicrobial activity*

Siliceous surfaces	Man-made fibers	Metals
Glass	Acrylic	Aluminum
Glass wool	Modacrylic	Stainless steel
Sand	Polyester	Galvanized metal
Stone	Cellulose acetate	
Ceramic	Rayon	Miscellaneous
	Acetate	Leather
Natural fibers	Anidex	Wood
Cotton	Spandex	Rubber
Wool	Vinyl	Plastic
Linen	Dacron	Formica
Felt	Viscose	

TABLE 4. *Microorganisms susceptible to Si-QAC*

Bacteria (gram positive)	Algae
Staphylococcus aureus	*Cyanophyta* (blue-green) *oscillatoria*
Streptococcus faecalis	*Cyanophyta* (blue-green) *anabaena*
Bacillus subtilis	*Chrysophyta* (brown)
	Chlorophyta (green) *Selenastrum gracile*
Bacteria (gram negative)	*Chlorophyta* (green) *Protococcus*
Salmonella chloeraesius	
Salmonella typhosa	Fungi
Escherichia coli	*Aspergillus niger*
Mycobacterium tuberculosis	*Aspergillus flarres*
Pseudomonas aeruginosa	*Aspergillus terreus*
Aerobacter aeiogenes	*Aspergillus verrucaria*
	Chaetomium globosum
Yeast	*Penicillum funiculosum*
Saccharomyces cerevisiae	*Trichophyton interdigital*
Candida albicans	*Pullularia pullulans*
	Trichoderm sp. *madison* P-42
	Cephaldascus fragans

treated surfaces without chemical adulteration of the solution also appears feasible. The use of alkoxysilanes of this nature may be useful in defining the site and mechanism of action of antimicrobial agents.

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