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Carbohydrate Research 337 (2002) 2495–2499

CARBOHYDRATE RESEARCH

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Note

# Preparation and investigation of antibacterial carbohydrate-based surfaces☆

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Received 17 June 2002; accepted 26 August 2002

**Abstract**

Surfaces bearing carbohydrate units have been modified in a two-step process to incorporate functionalities (lipophilic with polycationic units) that bear antibacterial activity. The effectiveness of these modified surfaces for antibacterial action against a series of seven Gram-positive and Gram-negative bacteria are reported. © 2002 Elsevier Science Ltd. All rights reserved.

It has for some time been recognized that cationic surfactants bear antibacterial activity.[1–5] Investigations of structure–activity relationships have demonstrated that in addition to a cationic site, a significant lipophilic component of the surfactant is involved in optimization of activity. With simple alkylbenzyldimethylammonium chlorides in their action against *Pseudomonas aeruginosa*, the optimal length of the alkyl chain has been noted to be 14 carbon atoms.[3] Optimal activity for a variety of structural variations of the water-soluble cationic surfactants appears to occur when an alkyl chain of between 10 and 14 carbon atoms is present.[6–10]

The mechanism of action of such cationic surfactants on bacteria is understood to be one of electrostatic interaction and physical disruption, rather than interference with a metabolic pathway.[11] The cationic site of the agent is able to bind to anionic sites of the cell-wall surface. With a significant lipophilic component present, it is then able to diffuse through the cell wall and bind to the membrane. As a surfactant, it is able to disrupt the membrane and permit the release of electrolytes and nucleic materials, leading to cell death.

Beyond the fundamental construction of an antibacterial agent that expresses its activity in a nonspecific manner, the challenge has been to impart such activity to a surface from which the active agent is not readily released. The binding of quaternary ammonium sites to glass surfaces through the use of silyl ether linkages was found to impart antibacterial activity to such surfaces.[12] Several polymeric surfaces have also been investigated, including polystyrene[13,14] and poly(propylene imine).[15]

In this light it appeared a reasonable possibility that other types of surfaces could be rendered antibacterial by the covalent attachment to them of polycationic units having lipophilic adjuncts. Prior efforts of our laboratory had demonstrated the facility with which such polyammonium units having lipophilic adjuncts could be prepared,[16,17] as well as the manner in which such materials could be attached to carbohydrate-derived sites in complex structures.[18] Thus, a variety of carbohydrate-derived surface materials were to be investigated for their potential to be rendered antibacterial by the binding to them of a polycationic adjunct.

For the present effort parent polyammonium units having lipophilic adjuncts were prepared by previously noted techniques[16,17] using 1,4-diazabicyclo[2.2.2]octane in reaction with appropriate haloalkanes in ethyl acetate solution (Scheme 1). The resultant monocationic salts (**1**–**8**), which readily precipitated from solution,

☆ Paper 13 in the series 'Polycations'.

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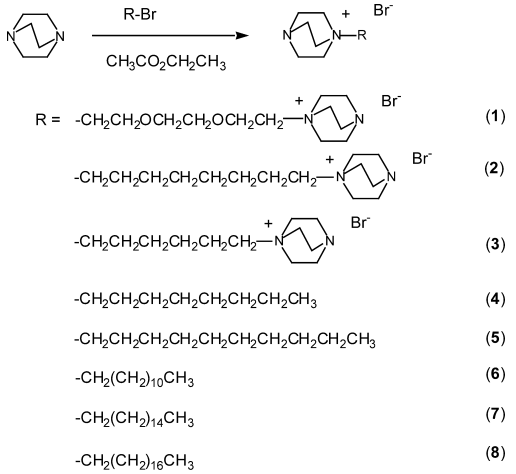
PII: S0008-6215(02)00316-6

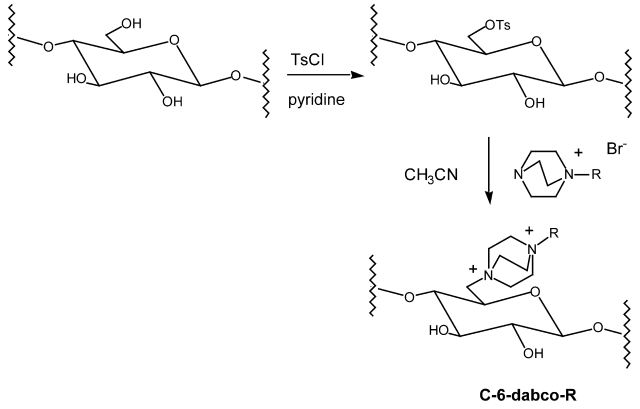
were isolated by suction filtration and dried under vacuum. All exhibited $^{1}H$ and $^{13}C$ NMR in accord with their proposed structures, as well as elemental analyses in accord with hydrated states of their elemental formulae, which have been reported previously.

Carbohydrate-based surfaces (100% cotton cloth, commercial grade; Whatman Grade 1 Chr) were activated and functionalized (as noted in Scheme 2), followed by washing and drying, to generate the modified surfaces as listed in Table 1.

The structural components presumed for the carbohydrate surfaces (as shown in Table 1 with designations relating to their structures) are deduced from the corresponding results previously found for attachment of the cationic components to cyclodextrins.[18] The reactivity of *p*-toluenesulfonyl chloride with alcohols is well known.[19] Primary hydroxyl sites can be functionalized quite specifically relative to secondary sites within the same molecule. Thus, tosylation (and subsequent reaction displacement by the tertiary amine) of the available hydroxyls at the 6-position of the carbohydrate units is presumed to occur without reaction also occurring at the available 2- and 3-position hydroxyl sites. (It is also




Scheme 1.




Scheme 2.

to be noted that tosylation of these systems can be accomplished without the use of pyridine. Such tosylation has been performed in acetonitrile medium with solid sodium carbonate present as an acid sink. The resultant modified carbohydrate materials exhibit the same activity as those produced using pyridine as a base.)

Seven bacterial strains (four Gram-negative and three Gram-positive) were investigated, as noted below, with note of their American Type Culture Collection reference number: *Escherichia coli* (ATCC # 14948), *Enterobacter aerogenes* (ATCC # 13048), *Enterobacter cloacae* (ATCC # 13047), *Proteus vulgaris* (ATCC # 13315), *Bacillus cereus* (ATCC # 14579), *Micrococcus luteus* (ATCC # 9341), and *Staphylococcus aureus* (ATCC # 6538).

These bacteria were added to the modified surfaces placed on a rich medium and incubated, along with three control experimental runs on the same plate. Growth of bacteria off the edge of the surface was observed visually, following which the bacteria and their remains yet on the surface were washed into 2 mL of liquid growth medium and incubated for 16 h. Growth of bacteria in these liquid media were measured turbidimetrically.

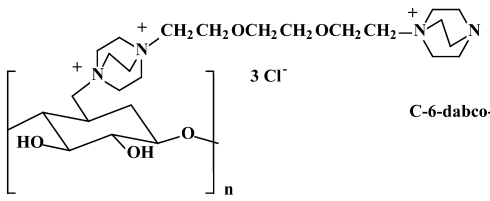
In all instances, involving each of the three types of carbohydrate-based surface and each of the lipophilic adjuncts, the control experiments exhibited full growth of the bacteria in the initial growth studies.

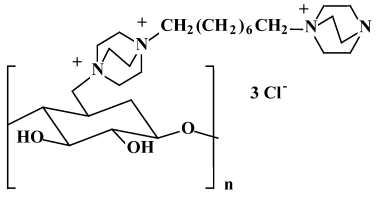
Bacterial growth on the modified surface and in the surrounding region with the modified surfaces was noted to be completely absent *only* in the sets of bacteria/modified surface noted below with each of the bacteria. Further, continued growth as measured turbidimetrically was noted to be zero as well for each of the following systems: *Escherichia coli*, C-6-dabco-C16; *Enterobacter aerogenes*, C-6-dabco-C16; *Enterobacter cloacae*, C-6-dabco-C12, and C-6-dabco-C16; *Proteus vulgaris*, C-6-dabco-C12, C-6-dabco-C16, and C-6-dabco-C18; *Bacillus cereus*, C-6-dabco-C10, C-6-dabco-C12, C-6-dabco-C16, and C-6-dabco-C18; *Micrococcus luteus*, C-6-dabco-C10, C-6-dabco-C12, C-6-dabco-C16, and C-6-dabco-C18; *Staphylococcus aureus*, C-6-dabco-C10, C-6-dabco-C12, C-6-dabco-C16, and C-6-dabco-C18.

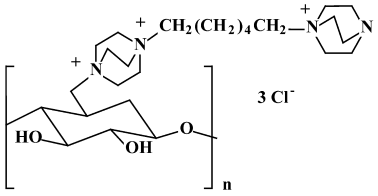
Full data for these continued growth experiments are summarized in Table 2. It should be noted that while bacterial growth was noted in the turbidity experiments with each of the types of surfaces with each of the other lipophilic adjuncts, zero growth was observed for the above-noted lipophilic adjuncts with the noted bacteria on each of the treated surfaces. Clearly, no viable bacteria survived exposure to these surfaces.

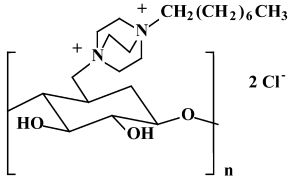
Fundamentally, this work demonstrates that broad antibacterial activity can be imparted to carbohydrate

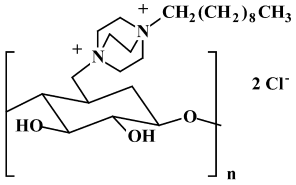
Table 1
Surface structure of carbohydrate-derived materials synthesized

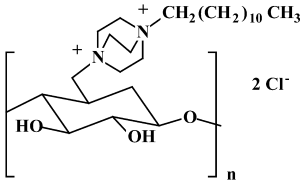


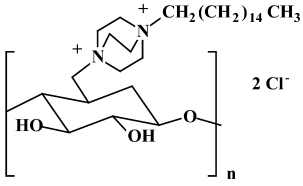



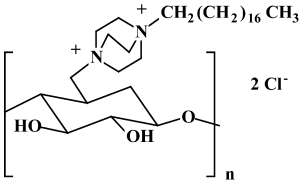




surfaces through the covalent attachment of cationic agents with lipophilic adjuncts. Modified surfaces C-6-dabco-C2OC2OC2-dabco, C-6-dabco-C8-dabco, C-6-dabco-C6-dabco, and C-6-dabco-C8 exhibited minimal (if any) antibacterial activity. Modified surfaces C-6-dabco-C2OC2OC2-dabco, C-6-dabco-C8-dabco, and C-6-dabco-C6-dabco involve a chain emanating from a cationic attachment site at the surface of the cloth and terminating in a polar and cationic site; modified surface C-6-dabco-C8 involves a relatively short lipophilic chain emanating from the cationic surface site. Clearly, the presence of a sufficiently long (10 methylene groups, minimum) completely lipophilic chain attached to the surface cationic site is required for activity.

Further, repeated ($10\times$) exposure of a tested modified surface to increasing amounts ($10\times$) of added bacteria produced the same result; the surfaces continued to exhibit the total antibacterial effect. This provides a particular application advantage for this type of system in that the antibacterial agent is not changed in the process, remains attached to the surface, and retains its activity.

A noteworthy, unanticipated, result of this investigation is the observation of an optimal chain length of the lipophilic unit for activity. Maximal antibacterial activity is observed toward both Gram-negative and Gram-positive bacteria with a chain length of 16 carbons in the lipophilic portion. We are continuing investigation of variations in nature of the lipophilic chain in an effort to understand this structure–activity relationship.

The antibacterial activity may be understood as occurring in a stepwise manner. The lipophilic chains can be subsumed by the bacterial species to a stage where the cationic portion is brought into intimate contact with the cell surface, and is subsumed sufficiently far that it is not easily expelled. Detergent-like action then results in cell surface disruption initiating cell destruction.

A particular advantage of such action is the lack of consumption of the antibacterial agent; it is not changed in the process and remains attached to the surface.

We are continuing with efforts: (1) to broaden the range of surfaces that can be so modified; (2) to seek optimal structure for the antibacterial agent; and (3) expand the library of microorganisms susceptible to such activity.

## 1. Experimental

*General procedure for the preparation of modified surfaces.*—A strip (2'' × 10'', 1.2 g, maximally 7.4 mmol of free primary hydroxyl) of the raw surface material (100% cotton cloth, commercial grade; Whatman Grade 1 Chr) was activated by addition to a

Table 2
Percentage growth of bacteria (as compared to blank) in liquid growth medium upon incubation following exposure to modified surface material

| | *B. cereus* | *M. luteus* | *S. aureus* | *E. coli* | *E. aerogenes* | *E. cloacae* | *P. vulgaris* |
|---|---|---|---|---|---|---|---|
| C-6-dabco-C2OC2OC2-dabco | 111 | 67 | 97 | 122 | 104 | 103 | 91 |
| C-6-dabco-C8-dabco | 82 | 139 | 97 | 110 | 103 | 91 | 100 |
| C-6-dabco-C6-dabco | 91 | 145 | 101 | 103 | 107 | 99 | 101 |
| C-6-dabco-C8 | 101 | 80 | 99 | 100 | 102 | 93 | 92 |
| C-6-dabco-C10 | 0 | 0 | 0 | 101 | 92 | 79 | 69 |
| C-6-dabco-C12 | 0 | 0 | 0 | 81 | 97 | 0 | 0 |
| C-6-dabco-C16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C-6-dabco-C18 | 0 | 0 | 0 | 47 | 85 | 20 | 0 |

solution containing an excess of *p*-toluenesulfonyl chloride (10 g, 52.5 mmol) in pyridine (25 mL). After standing in the stirred solution for 4 h, the strip was removed and washed with water chilled with ice. Attachment of the polycationic ligand was then accomplished by placement in a stirred acetonitrile solution (25 mL) containing the monocation unit (**1**–**8**) having lipophilic adjunct (an excess, e.g., with **7** to generate C-6-dabco-C16, 20 g, 54 mmol) and agitated for 24 h. After this time the modified surface material was removed from the reaction medium and washed repeatedly with water, followed by a brine wash. It was finally dried in air without heating.

*General procedure for determination of the antibacterial characteristics of the modified surfaces.*—The rich medium used for bacterial growth was prepared from Bacto-tryptone, Bacto-agar, yeast extract and sodium chloride. Each modified surface sample was investigated for its antibacterial effect with each of the bacteria studied in a four-part experiment. Specifically, on the same plate, bearing the growth medium, were placed four separate experimental runs, those being:

**A**, untreated surface material, to which no bacteria had been added;

**B**, surface material that had been subjected to the solvent washing procedures of reaction but without addition of the reagent materials, to which the bacteria being investigated were added. In each instance 5 μL of the stock dispersion of bacteria in log growth phase were added using an Oxford Benchmate Pipetman, placing the entire load of bacteria at the center of the test swatch of material (square, 0.5'' to a side). For the strains investigated, this corresponded to addition of the following number of bacteria: *Escherichia coli*, $1.41 \times 10^5$; *Enterobacter aerogenes*, $2.14 \times 10^5$; *Enterobacter cloacae*, $2.56 \times 10^4$; *Proteus vulgaris*, $2.34 \times 10^5$; *Bacillus cereus*, $1.54 \times 10^5$; *Micrococcus luteus*, $3.20 \times 10^5$; *Staphylococcus aureus*, $1.47 \times 10^5$.

**C**, untreated surface material, to which the bacteria being investigated were added in the same manner and amount as noted for **B** above;

**D**, modified surface material, to which the bacteria being investigated were added in the same manner and amount as noted for **B** above.

The growth plate holding the four experiments was incubated overnight at 35 °C. Growth was noted visually in the region around the material surface. Subsequently, the surface material was removed from the growth medium and placed in 4 mL of fresh growth medium and incubated at 35 °C for 16 h. Growth of bacteria in this instance was measured turbidimetrically using a Beckman Model 25 UV–vis spectrophotometer at 60 nm.

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