

Model Studies on Dental Plaque Formation: Deoxyhexoses in *Actinomyces viscosus*†

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A careful examination of two strains of *Actinomyces viscosus*, T14V (virulent) and T14AV (avirulent), revealed no qualitative or quantitative difference in 6-deoxyhexose content of their cell surface. For a further study of the role of these sugars in cell surface-related phenomena, the stereochemical configuration of deoxyhexoses of *A. viscosus* T14 was established by two complementary approaches. (i) Examination of the biosynthetic pathway was found to lead to the formation of both 6-deoxy-L-talose and 6-deoxy-L-mannose and showed no differences in the ability of either bacterial strain, *A. viscosus* T14V or T14AV, to produce the precursors of these cell wall components. The biosynthetic pathway for 6-deoxy-L-talose and 6-deoxy-L-mannose was found to originate from deoxythymidine diphosphate (dTDP)-D-glucose, which in turn is converted to dTDP-4-keto-6-deoxy-D-glucose. Epimerization at carbons 3 and 5 of the hexose moiety of dTDP-4-keto-6-deoxy-D-glucose is followed by stereospecific reduction with reduced nicotinamide adenine dinucleotide phosphate to yield dTDP-6-deoxy-L-talose and dTDP-6-deoxy-L-mannose. In cell-free extracts of both *A. viscosus* T14 and T14AV, an identical ratio of 6-deoxy-L-talose to 6-deoxy-L-mannose of 1:8 was produced. Known precursors for the D-isomers of the same 6-deoxyhexoses such as guanosine diphosphate-D-mannose and dTDP-D-mannose were not converted by *A. viscosus* T14 cell-free extracts. (ii) Isolation of 6-[U-¹⁴C]deoxytalose and 6-[U-¹⁴C]deoxymannose from both strains of *A. viscosus* T14 was carried out by growing cells in a medium containing D-[U-¹⁴C]glucose. Again no qualitative or quantitative difference was noticeable between the two strains when 6-deoxyhexoses were released from whole cells or purified cell walls by acid hydrolysis. Radioactive 6-[U-¹⁴C]deoxytalose isolated from the cell surface was used in an isotope dilution experiment to establish the stereochemical configuration of this 6-deoxyhexose. The radioactive sugar was mixed with unlabeled standard D- or L-6-deoxyhexose, respectively, and conversion to the corresponding 1-phenylflavazole derivative was carried out. Recrystallization to constant specific activity identified the radioactive sugar isolated from *A. viscosus* to be the L-isomer. A facile synthesis of the rare sugars 6-deoxy-L-talose and 6-deoxy-D-talose is reported.

Considerable information has accumulated to implicate microorganisms of the oral cavity in dental plaque formation that can lead to dental caries, periodontal disease, and gingivitis. Experimental evidence indicates that different bacterial species are found to be localized within specific sites of the oral cavity (7). Numerous

studies have shown that the ability of oral microorganisms to adhere to various surfaces within the mouth correlates well with their proportions found indigenously (10). From these observations it appears that the ability of bacteria to adhere to a surface must be related to a specific interaction with the cell surface. This ability is a prerequisite for colonization of bacteria in open systems like the oral cavity. Several reports indicate that strains of *Actinomyces* are involved in the initiation of dental plaque formation and were demonstrated to be related to root surface caries as well as to periodontal disease (15). In addition, it was shown that plaque formation may occur even in the absence

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of sucrose with microorganisms like *Actinomyces viscosus* (15).

Hammond and co-workers have studied two strains of *Actinomyces viscosus*, T14V (virulent) and T14AV (avirulent), and reported the occurrence of a specific virulence-associated antigen containing a high proportion of the unusual sugar 6-deoxy-L-talose (8). However, no evidence for the assignment of the L-configuration of this sugar was provided, although various microorganisms were shown to produce the D-isomer of 6-deoxytalose (17) as well as its 4-epimer 6-deoxy-D-mannose (D-rhamnose) (9).

Various other investigators have examined the same two strains of *A. viscosus* with the objective of demonstrating unique and specific cell wall components of the virulent strain. More recently, several independent reports indicate the occurrence of fine surface fibrils (D. C. Birdsell and W. Fischlschweiger, *J. Dent. Res.* 56: B117, Abstract 263, 1977), which are found to be more abundant on the T14V cell wall than on the T14AV cell wall (3).

We examined qualitative and quantitative differences in the occurrence of cell wall-bound 6-deoxytalose and 6-deoxymannose in *A. viscosus* T14V and T14AV, as well as the biosynthetic ability of the two strains to produce deoxyhexoses. For a detailed study of the role of these sugars in cell surface-related phenomena, the stereochemical configuration of deoxyhexoses will be established.

(Preliminary reports of this work have appeared [C. A. Tylenda and F. P. Lombardi, *Fed. Proc.* 34:535, 1975; C. A. Tylenda and O. Gabriel, *Fed. Proc.* 35:1701, 1976].)

MATERIALS AND METHODS

Growth and maintenance of bacterial cells. *A. viscosus* T14V and T14AV were generous gifts obtained from B. F. Hammond (University of Pennsylvania). Cells were stored on Trypticase soy broth (BBL) agar slants at 4°C for short periods. Frozen and lyophilized cells were kept at -20°C. Cells were grown on Trypticase soy broth at 37°C for 24 and 48 h, depending on the inoculum used, and were harvested in stationary phase.

Escherichia coli O45 (a gift of K. B. Jann, Max Planck Institute, Freiburg, Germany) and *E. coli* B (American Type Culture Collection) were stored refrigerated on nutrient agar slants (Difco) and grown in antibiotic medium no. 3 (Difco). Cells were harvested in midlog phase, and were washed once with water and used immediately or stored at -20°C until needed.

Preparation of extracts used for enzymatic assays. *A. viscosus* T14V or T14AV cells were suspended in 25 mM tris(hydroxymethyl)aminomethane buffer (Sigma) (pH 8.0) containing 10 mM β -mercaptoethanol (Sigma), 1 mM ethylenediaminetetraacetic acid, and 1 mM MgSO₄ in the following proportions: 1 g (wet weight) of cells to 3 ml of buffer to 1 g of glass

beads. A 10-ml sample of cell suspension was sonically disrupted in an ice bath at 70 to 80 W (Heat Systems-Ultrasonics, Inc., model W185 D) in 1-min pulses for 10 to 15 min with appropriate cooling intervals. The temperature remained below 15°C throughout the experiment. The cell suspension was decanted and centrifuged at 12,000 $\times g$ (Sorvall Refrigerated Centrifuge, RC2-B) for 20 min. The supernatant was assayed for oxidoreductase activity. An alternate procedure to obtain a preparation capable of catalyzing the reactions leading to formation of deoxythymidine diphosphate (dTDP)-4-keto-6-deoxy-D-glucose was carried out by freezing and thawing cells. For this purpose, 1 g of frozen cells was suspended in 1 ml of the buffer described above. The cell suspension was immediately used for incubation with substrate as indicated below.

Preparation of cell-free extracts from *E. coli* O45. *E. coli* O45 cells (1 g, wet weight) were suspended in 10 ml of tris(hydroxymethyl)aminomethane buffer containing mercaptoethanol, ethylenediaminetetraacetic acid, and MgSO₄ as described above. Four 10-s bursts of sonic disruption were necessary for complete rupture of cells. The suspension was centrifuged at 12,000 $\times g$ for 10 min, and the supernatant was used for enzymatic incubation experiments.

Preparation of sugar nucleotides. dTDP-D-mannose was prepared from deoxythymidine monophosphate and D-mannose-1-phosphate by the Michelson procedure (13). dTDP-D-[U-¹⁴C]glucose was prepared by enzymatic synthesis by a cell-free extract of *Pseudomonas aeruginosa* ATCC 7700 (11).

Assay for dTDPG oxidoreductase activity. The incubation mixture contained 50 to 200 μ l of cell-free extract, 0.1 μ mol of deoxythymidine diphosphogluconate (dTDPG; Sigma), and 50 μ l of the tris(hydroxymethyl)aminomethane buffer described above. Total volume ranged from 125 to 175 μ l. Control tubes contained no dTDPG. Nicotinamide adenine dinucleotide (NAD; 0.05 μ mol; Boehringer-Mannheim) was added to the tubes containing *A. viscosus* cell-free extract and omitted from the tubes containing *E. coli* O45 cell-free extract. Tubes were incubated in a water bath at 37°C for 15 min, then transferred to an ice bucket. Water was added to bring the total volume to 1 ml, and the optical density at 318 nm was read. The conversion of dTDPG to dTDP-4-keto-6-deoxy-D-glucose was determined by adding 10 μ l of 10 N NaOH and measuring the increase in optical density at 318 nm. A maximum value was reached after 10 to 15 min (14).

dTDPG oxidoreductase activity was also monitored in freeze-thaw extracts. A 100- μ l cell suspension was used, and incubation time was 1 h. Total volume was adjusted to 0.5 or 1.0 ml, and samples were placed in a boiling water bath for 1 min. After centrifugation the supernatants were assayed for dTDP-4-keto-6-deoxy-D-glucose as described above.

Chemical reduction of the dTDP-4-keto-6-deoxy-D-glucose intermediate. Reduction of the dTDP-4-keto-6-deoxy-D-glucose intermediate was accomplished by the addition of NaBH₄. dTDP-D-[U-¹⁴C]glucose was incubated with cell-free extract or freeze-thaw extract as described above. A 10- μ l sample of a 1 M solution of NaBH₄ in 10 mM NaHCO₃ was added, every 10 min at room temperature, four times.

The pH was adjusted to 2 with HCl, and the tubes were placed in a boiling water bath for 10 min. After centrifugation the supernatant was passed through an Amberlite MB-3 mixed-bed resin column. The eluate was reduced to dryness, 0.5 ml of methanol was added, and the sample was reduced to dryness. The addition and evaporation of methanol was repeated three times to remove any traces of borate. Sugars were analyzed by paper chromatography.

Enzymatic reduction of dTDP-4-keto-6-deoxy-D-glucose. The enzymatic conversion of dTDP-4-keto-6-deoxy-D-glucose to dTDP-6-deoxy-L-talose and dTDP-6-deoxy-L-mannose was achieved by the addition of reduced NAD phosphate (NADPH) to the incubation mixture. dTDP-D-[U-¹⁴C]glucose was incubated with a cell-free extract as described earlier. A 10- μ l volume of 50 mM NADPH was added initially, then every 30 min for 2 h (2 μ mol total addition). The pH was adjusted to 2 with HCl, and the mixture was placed in a boiling water bath for 10 min. Denatured proteins were removed by centrifugation. The pH was adjusted to 8 with NH₄OH, alkaline phosphatase was added, and the mixture was incubated at 37°C for 15 min. The mixture was passed through an Amberlite column, and the eluate was concentrated and spotted on chromatography paper. Sugars were separated as described earlier. Radiolabeled sugars were eluted from the paper with water.

Chemical synthesis of 6-deoxy-L-talose. To provide an authentic standard for the identification of the sugar 6-deoxytalose occurring in the cell wall of *A. viscosus*, 6-deoxy-L-talose was prepared by a modification of a procedure originally described by Collins and Overend (4). This procedure involves inversion of carbon 2 configuration of methyl-3,4-O-isopropylidene- α -L-fucopyranoside. The oxidation of this intermediate to give methyl-3,4-O-isopropylidene-6-deoxy- α -L-lyxohexopyranose-2-ulose in good yield was carried out with RuO₄ in CCl₄ (2). Catalytic reduction of the keto intermediate with PtO₂, followed by acid hydrolysis, ultimately afforded crystalline 6-deoxy-L-talose, mp 127°C (Fig. 1).

It should be noted that 6-deoxy-D-talose is prepared by an analogous reaction sequence using methyl-3,4-O-isopropylidene- α -D-fucopyranoside as the starting material.

Preparation of cell walls. Lyophilized *A. viscosus* T14V or T14AV cells (500 mg) were suspended in 10 ml of deionized water to which 3 g of acid-washed glass beads was added (0.1-mm diameter). The cells were ruptured by sonic disruption (Heat Systems, Ultrasonics) with intermittent pulses in an ice bath. The isolation of cell walls was carried out according to the procedure of Wallinder and Neujahr (16).

Paper chromatography. Sugars were separated on Whatman no. 1 chromatography paper using pyridine-ethyl acetate (1:3.6, water saturated) solvent system no. 1. Sugar standards, with the exception of 6-deoxytalose, were obtained commercially. 6-Deoxytalose, for use as a standard, was obtained by enzymatic conversion of dTDP-D-glucose to dTDP-6-deoxy-L-talose by a cell-free extract of *E. coli* O45 or by organic synthesis. Radiolabeled sugars were located by radiochromatogram scan (Packard model 7201) and by silver nitrate dip (1).

Gas-liquid chromatography. Carrier sugars were added to the ¹⁴C-labeled sugars. Two derivatives were prepared: trimethylsilyl ethers and alditol acetates. Sugars were first reduced to their corresponding alcohols by using NaBH₄. To 100 μ l of water containing 0.1 to 10 μ mol of sugar, an aliquot of 1 M NaBH₄ in 10 mM NaHCO₃ was added every 10 min three times to give 50-fold molar excess. Na⁺ was removed by passing the mixture through an AG50W-X4 (Bio-Rad) column. Borate ions were removed by the addition of 0.5 ml of methanol and evaporation to dryness, a procedure that was repeated three times.

(i) **Trimethylsilyl ethers.** To 1 μ mol of sugar alcohol in pyridine, 100 μ l of bis-trimethylsilylacetamide containing bis-trimethylchloride as a catalyst was added. After 10 min at room temperature, the pyridine was removed, and the sample was taken up in hexane prior to injection into the column.

(ii) **Alditol acetates.** Acetic anhydride (100 μ l) was added to 1 μ mol of sugar alcohol in pyridine and heated at 100°C for 3 h. The pyridine was removed, and the sample was taken up in hexane.

A 6-foot (ca. 1.83 m) column containing 3% SE-52 resin on 80- to 100-mesh acid-washed Chromosorb 2 in a Hewlett-Packard model 5750 research chromatograph was used for analysis. The instrument was equipped with a stream splitter. The material that

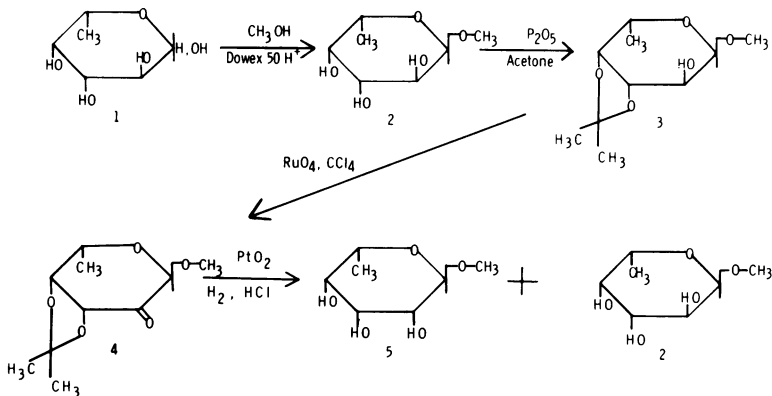


FIG. 1. Reaction scheme for the synthesis of 6-deoxy-L-talose.

eluted from the stream splitter was collected in fractions. The position of the radiolabel was correlated with the position of the peak detected by flame ionization. Co-chromatography of trace amounts of radiolabeled sugars with authentic carrier sugars was demonstrated.

RESULTS

Formation of dTDP-4-keto-6-deoxy-D-glucose. Incubation of dTDP-[U - ^{14}C]glucose was carried out with either cell-free extracts of *A. viscosus* T14V and T14AV or cells after freezing and thawing. In each instance, the incubation led to quantitative conversion of dTDP-[U - ^{14}C]glucose to a new product. Two independent methods were used to establish the formation of dTDP-4-keto-6-D-glucose: (i) the characteristic ultraviolet spectrum with a peak at 318 nm after addition of alkali (14); and (ii) reduction of the product with sodium borohydride, yielding a mixture of dTDP-6-deoxy-D-[U - ^{14}C]glucose and dTDP-6-deoxy-D-[U - ^{14}C]galactose. The mixture of sugar nucleotides was subjected to acid hydrolysis (0.01 N HCl for 10 min at 100°C), and the radioactive sugars were separated by paper chromatography in solvent no. 1. Location of the monosaccharides of the chromatogram by scanning for radioactivity as well as by their reducing property (1) revealed the presence of the expected 4-epimers 6-deoxy-D-glucose and 6-deoxy-D-galactose as the sole products.

The enzymatic activity to convert dTDP-D-glucose to dTDP-4-keto-6-deoxy-D-glucose was demonstrable in both the virulent and avirulent strains of *A. viscosus* T14. This enzymatic activity was also demonstrable in both strains by the methods just described after freezing and thawing of cells.

In separate experiments, analogous incubations were carried out using guanosine diphosphate-D-glucose and dTDP-D-mannose as a substrate. No experimental evidence for the formation of the corresponding nucleoside diphosphate-4-keto-6-deoxy-D-mannose was observed.

Enzymatic conversion of dTDP-4-keto-6-deoxy-D-glucose to dTDP-6-deoxyhexoses. The addition of NADPH to an incubation mixture containing dTDP-D-glucose and *A. viscosus* cell-free extract results in the formation of dTDP-6-deoxy-L-talose and dTDP-6-deoxy-L-mannose (dTDP-L-rhamnose). The sugars were isolated by procedures described above. In each case, identification of each sugar was verified by two independent methods: (i) the comigration of the experimentally produced radiolabeled sugar with the corresponding unlabeled authentic sugar standard on paper chromatography in solvent no. 1 and (ii) the comigration of the trimethylsilyl ether derivative of the reduced, experimentally produced, radiolabeled sugar with the same derivative of reduced unlabeled sugar

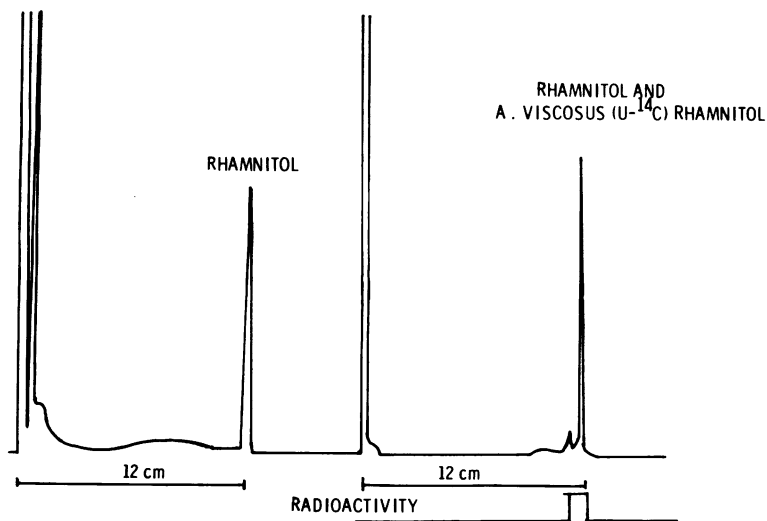


FIG. 2. Gas chromatograms of the trimethylsilyl ether derivative of 6-deoxymannitol (rhamnitol). The chromatogram on the left shows the retention time of the standard reduced sugar derivative. The chromatogram on the right shows the retention time of the derivative of a mixture of reduced unlabeled sugar standard and a trace amount of 6-[U - ^{14}C]deoxymannose from *A. viscosus* T14. The radiolabeled sugar was obtained by mild acid hydrolysis of *A. viscosus* T14 whole cells grown in Trypticase soy broth containing D-[U - ^{14}C]glucose (specific activity = 4 μ Ci/ml of medium). 6-[U - ^{14}C]deoxymannose was separated by paper chromatography in pyridine-ethyl acetate (1:3.6, water saturated), located by radiochromatogram scan, and isolated by elution from the paper.

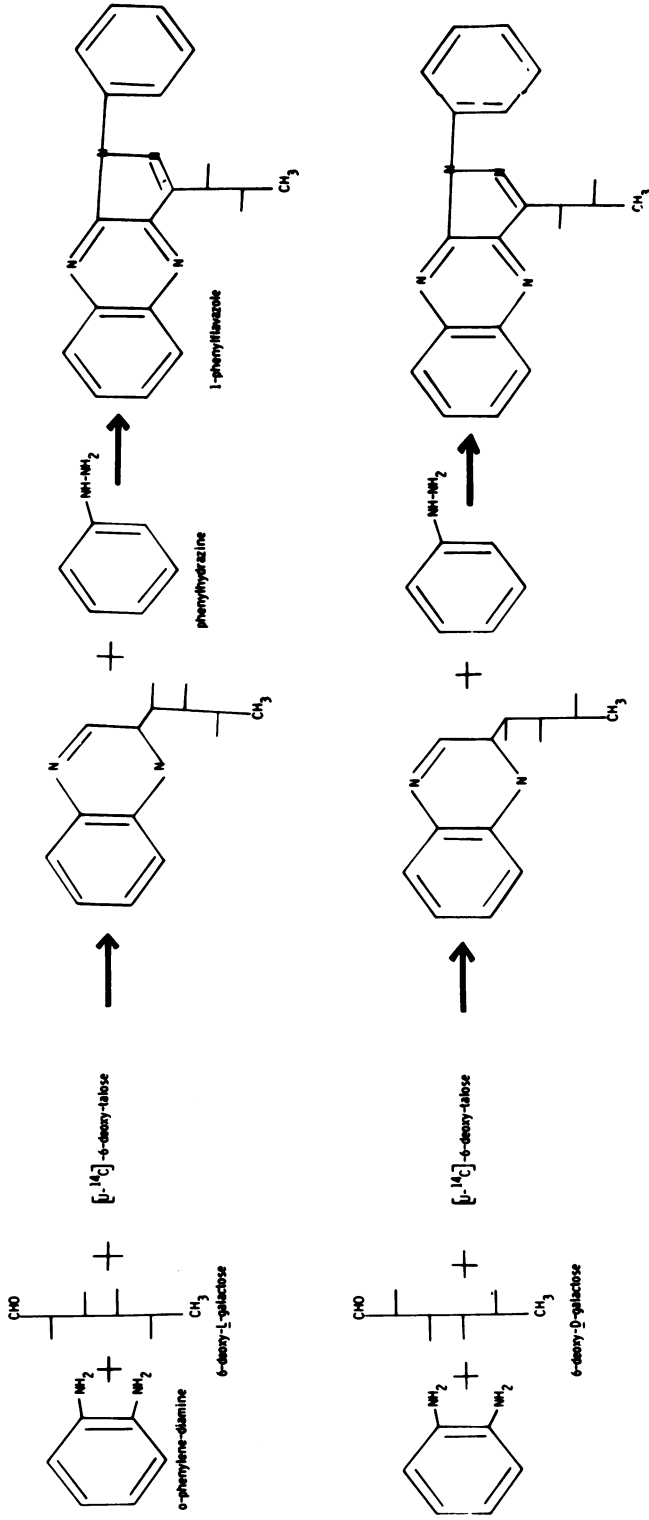


FIG. 3. Reaction scheme for the conversion of 6-[U-¹⁴C]deoxytalose isolated from the cell wall to its 1-phenylflavazole derivative. Addition of unlabeled carrier 6-deoxy-D-galactose or 6-deoxy-L-galactose was as described in the text.

standard on gas chromatography. This is illustrated in Fig. 2 for 6-deoxymannose.

The ratio of dTDP-6-deoxy-L-talose to dTDP-6-deoxy-L-mannose produced by incubation of dTDP-D-glucose with *A. viscosus* cell-free extracts in the presence of NADPH was approximately 1:8 for both strains.

Stereochemical configuration of 6-deoxytalose and 6-deoxymannose isolated from the cell walls. Preparatory to hapten inhibition studies with cell wall antibodies directed against carbohydrate-containing cell wall components, we decided to establish the stereochemical configuration of cell wall-associated deoxyhexoses.

For this purpose, radiolabeled *A. viscosus* cells were prepared by growing cells in TSB, to which a radiolabeled precursor was added. For the preferential labeling of carbohydrates, D-[U-¹⁴C]glucose (Amersham/Searle) was added to a specific activity of 0.5 μ Ci/ml of medium. Either a cell wall preparation, as described in Materials and Methods, or cells without prior isolation of cell walls can be used. The unique location of 6-deoxyhexoses exclusively as cell wall components and the very low steady-state concentration of the precursors make this simplified procedure feasible. In this instance, cells were harvested and washed twice in ice-cold water. The washed cells, or a cell wall preparation, were directly subjected to hydrolysis in 1 N HCl at 100°C for 2 h. Residual material was removed by centrifugation, and the sample was taken to dryness in vacuo and passed through an Amberlite MB-3 column. The eluate was subjected to paper chromatography in solvent no. 1. Approximately 1 μ mol of 6-deoxytalose containing 1.2×10^5 dpm was isolated chromatographically pure. The radioactive sample was divided into two equal parts. One part was diluted with 300 μ mol of authentic unlabeled 6-deoxy-L-galactose (L-fucose), and to the second aliquot 300 μ mol of the D-isomer 6-deoxy-D-galactose (D-fucose) was added. The final specific activity for each sample was determined to be 2,000 dpm/ μ mol. 6-Deoxyhexose content was measured with the cysteine sulfuric acid reaction (5). Each aliquot was then individually converted to its 1-phenylflavazole according to Fig. 3, following a procedure previously employed in this laboratory (6). It should be noted that it is possible to use fucose instead of 6-deoxytalose in this radioisotope dilution experiment, since only the centers of chirality at carbons 4 and 5 are retained when the 1-phenylflavazole derivative is formed. Consequently, 6-deoxytalose and 6-deoxygalactose yield the same 1-phenylflavazole derivative. Repeated recrystallization and determination of specific activity after each recrystallization were

carried out. The absorption coefficients, $E_{333} = 8.99 \times 10^3$ and $E_{409} = 3.38 \times 10^3$, of the derivative in ethanol were used for quantitative determination. The counting of individual recrystallized samples was carried out in samples containing at least 1,000 cpm and appropriate quench corrections. After six recrystallizations, the specific activity of the D-isomer decreased with each recrystallization, while that of the L-isomer remained constant close to the specific activity of the starting material (2,000 dpm/ μ mol).

DISCUSSION

A comparative study of cell wall-associated deoxyhexoses and their biosynthetic pathway was carried out in *A. viscosus* T14V and T14AV. The absence of significant differences in 6-deoxyhexose content could be interpreted to indicate that our strain lost virulence during long-term cultivation in our laboratory. Several precautions were taken to safeguard the examination of *A. viscosus* T14V and T14AV instead of genetic variations of these strains. Lyophilized stock cultures were kept, and none of these cultures was transferred for more than 6 weeks. The cultures were analyzed for their proper biochemical functions, such as production of extracellular polysaccharide for the avirulent strain. In addition, cultures were monitored by a specific agglutination test with *Streptococcus sanguis* 34 that will agglutinate the virulent strain but not the avirulent strain (12). According to the above criteria, our virulent strain was found to be indistinguishable from that kept by John O. Cisar (National Institute for Dental Research, Bethesda, Md.).

Cell-free extracts from either *A. viscosus* T14V or T14AV were incubated with dTDP-D-glucose and converted to a mixture of dTDP-6-deoxy-L-mannose and dTDP-6-deoxy-L-talose with the intermediate formation of dTDP-4-keto-6-deoxy-D-glucose. The reaction sequence is shown in Fig. 4.

As can be seen, dTDP-D-glucose is converted to dTDP-4-keto-6-deoxy-D-glucose. This intermediate is epimerized at carbons 3 and 5, followed by a reduction step that requires the participation of NADPH. According to this reaction scheme, dTDP-D-glucose is the precursor for the L-isomers of 6-deoxymannose and 6-deoxytalose (6). By contrast, guanosine diphosphate-D-mannose was shown to lead to formation of the D-isomers of the same deoxyhexoses (17).

When cell-free extracts of both strains were incubated with guanosine diphosphate-D-mannose and dTDP-D-mannose, no conversion to the corresponding 4-keto intermediates was ob-

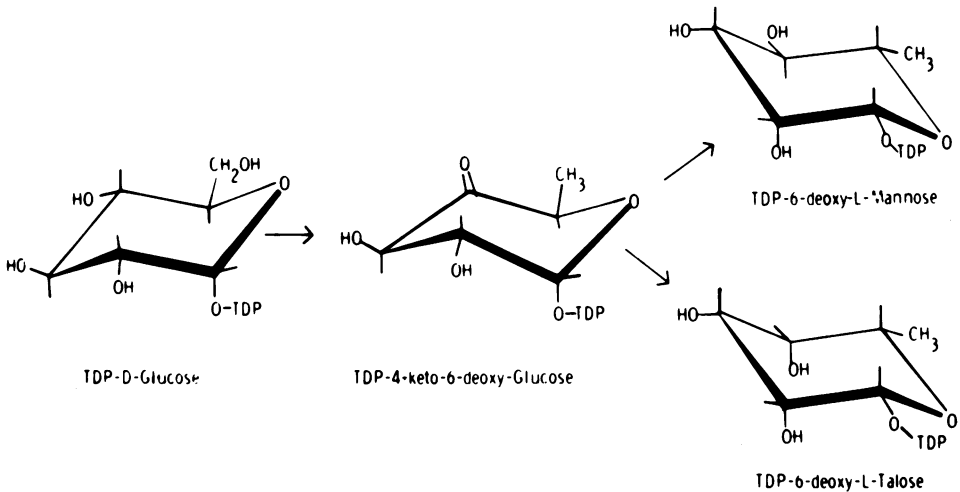


FIG. 4. Pathway for the biosynthesis of dTDP-6-deoxy-L-mannose and dTDP-6-deoxy-L-talose from dTDP-D-glucose in *A. viscosus* T14V and T14AV.

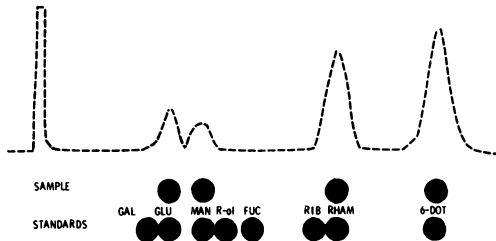


FIG. 5. Sugars released from *A. viscosus* T14V and T14AV cell walls by treatment with 1 N HCl for 2 h.

served. On the basis of these experiments, it was concluded that the 6-deoxyhexoses found in *A. viscosus* are the L-isomers.

Comparative examination of *A. viscosus* T14V and T14AV revealed that both strains produced about eight times more 6-deoxymannose than 6-deoxytalose in the in vitro incubation.

For the unambiguous identification of cell wall-bound 6-deoxyhexoses, cells were grown on a medium supplemented with D-[U - 14 C]glucose, and cell walls were prepared as indicated in Materials and Methods. Release of 6-deoxyhexoses by acid hydrolysis revealed the same pattern (Fig. 5) for both strains T14V and T14AV. 6-[U - 14 C]deoxytalose was isolated and converted to its 1-phenylflavazole derivative after addition of nonradioactive D- or L-isomer of carrier 6-deoxyhexose. Repeated recrystallization, followed by determination of specific activity, showed constant specific activity for the L-isomer and rapidly declining activity for the D-isomer. This establishes 6-deoxy-L-talose as the naturally occurring cell wall component in *A. viscosus* T14V and T14AV. We were unable to

detect any qualitative or quantitative differences in the 6-deoxyhexose content of the cell walls in *A. viscosus* T14V and T14AV.

Recent findings of other investigators indicate the occurrence of a "virulence-associated" antigen (V-antigen) in *A. viscosus* T14V (3). The presence of the V-antigen appears to be a quantitative rather than a qualitative difference and parallels the occurrence of fibrils on the cell surface of *A. viscosus* T14V and T14AV (3). Thus, the V-antigen as well as surface fibrils are present in both T14V and T14AV but to a much larger extent in T14V. Additional experimental evidence indicates that quantitative changes of antigens can occur depending on specific growth conditions employed (J. T. Powell and D. C. Birdsall, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, K189, p. 158).

It should be noted that the V-antigen does not contain 6-deoxytalose (3), and it seems to be primarily composed of proteins.

Our studies indicate no difference between strains T14V and T14AV in their content of 6-deoxy-L-mannose and 6-deoxy-L-talose. This eliminates these sugars as constituents of the V-antigen and is consistent with the notion reported by others that the V-antigen does not contain 6-deoxytalose (3). At present, we have no explanation for the disparity between these experimental facts and the findings reported by Hammond and co-workers (8), which indicate a direct correlation between 6-deoxytalose content and the V-antigen.

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