INFECTION AND IMMUNITY, Nov. 1979, p. 427-434
0019-9567/79/11-0427/08$02.00/0

Vol. 26, No. 2

Mannose-Contaminating Agglutinin for *Actinomyces viscosus* and *Actinomyces naeslundii*

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Received for publication 11 June 1979

Rapid agglutination of *Actinomyces viscosus* and *Actinomyces naeslundii* cells by D-mannose solutions was observed during studies of their attachment to mammalian cells in vitro. The specificity of the agglutination reaction was studied by slide agglutination tests and by measuring the rate of decrease in optical density of bacterial phosphate buffer suspensions caused by the settling of bacterial aggregates. *Actinomyces* cells were agglutinated by protein-containing mannose solutions of several chemical suppliers. Solutions of sugars other than D-mannose and solutions of mannitol and mannan all failed to agglutinate *A. viscosus* and *A. naeslundii*. "Mannose-enhanced" agglutination was impaired by boiling or autoclaving the mannose but was not affected by heating the bacteria, the presence of chloramphenicol, running the assay in the cold, or incorporating any of several commercially purchased sugars in the reaction mixture. During these hapten inhibition experiments, only 6-deoxy-L-talose-containing extracts of an *A. viscosus* strain retarded the rate of mannose-enhanced agglutination. Protein-containing fractions of D-mannose mother liquors also agglutinated cells of *A. viscosus* and *A. naeslundii*. Other species of oral gram-positive rods were not agglutinated by mannose solutions. Together the data indicate that plant seed-derived D-mannose contains a protein-associated agglutinin for *A. viscosus* and *A. naeslundii* which may function via a "lectin-like" selective affinity for the unique cell wall sugar 6-deoxy-L-talose.

Several investigations of host-parasite interactions have dealt with sugar specificity in bacterial attachment to human epithelial cells, erythrocytes and cultured mammalian cells (4, 16-18). Monosaccharides have been used often for these hapten inhibition studies to determine the nature of receptors with selective affinities for microorganisms. However, some commercial preparations of monosaccharides are known to contain hemagglutinating proteins which may interfere with receptor sites and cause inaccurate interpretation of data (13). Previous investigations in our laboratory (5-7) have concentrated on the intraoral ecology and specific surface attachment phenomena of *Actinomyces viscosus* and *Actinomyces naeslundii*, which are known to induce periodontal disease and root surface carious lesions in animals and are suspected in the etiology of these diseases in humans (10, 11, 20). While attempting to inhibit their attachment to epithelial cells and their hemagglutinating activity with various sugars, we observed that cells of *A. viscosus* and *A. naeslundii* formed large aggregates when exposed to D-mannose solutions. The present investigation tested the specificity of this agglutination reaction. Data indicating that commercially available D-mannose and by-products of its manufacture contain contaminating agglutinins for strains of *A. viscosus* and *A. naeslundii* are presented.

MATERIALS AND METHODS

Cultures and cultural conditions. Laboratory strains representing species of the genus *Actinomyces* and other genera of oral gram-positive rods used in the study are listed in Table 1. These strains were maintained by monthly transfer on slants of brain heart infusion agar (Difco Laboratories, Detroit, Mich.). An additional 80 strains of *A. viscosus* and *A. naeslundii* were freshly isolated from supragingival root surface plaque. Identification was confirmed by a combination of tests including agglutination of whole cells with sera specific for *A. viscosus* or *A. naeslundii*, analysis of acid end products by gas chromatography, and detection of the specific cell wall sugar 6-deoxy-L-talose (6-DOT) by paper chromatography of cell hydrolysates (E. D. Fillery, D. A. Grove, K. H. Chan and R. P. Ellen, Int. Assoc. Dent. Res., abstr. no. 1122, 1978). Fresh isolates were used within three subcultures on brain heart infusion slants of their primary

TABLE 1. *Agglutination of laboratory strains of A. viscosus, A. naselundii, and other species of oral gram-positive rods by* D*-mannose solutions*

Organism	Strain	Source[a]	Enhanced agglutination[b]
Actinomyces viscosus (human)	38fc	A	+
A. viscosus (human)	C12	A	+
A. viscosus (human)	T14B	A	−
A. viscosus (human)	M100	A	−
A. viscosus (human)	P2	A	+
A. viscosus (human)	11B2	B	−
A. viscosus (human)	A1353	B	+
A. viscosus (human)	H21	B	+
A. viscosus (human)	Be32	B	−
A. viscosus (human)	W1053	B	+
A. viscosus (human)	B236	B	−
A. viscosus (human)	B25	B	+
A. viscosus (human)	WVU627	BC	+
A. viscosus (rodent)	ATCC 15987	B	−
A. viscosus (rodent)	WVU440	B	+
A. viscosus (rodent)	A1231	B	−
Actinomyces naeslundii (human)	C2	A	+
A. naeslundii (human)	C10	A	+
A. naeslundii (human)	ATCC 12104	B	+
A. naeslundii (human)	B120	B	+
A. naeslundii (human)	4A05	B	+
A. naselundii (human)	W752	B	+
A. naselundii (human)	TF11	B	+
A. naselundii (human)	WVU398A	C	+
A. naselundii (human)	631	C	−
Actinomyces odontolyticus	ATCC 17982	A	+ (Slowly)
Actinomyces israelii	ATCC 10048	A	−
A. israelii	9057	C	−
A. israelii	1119	C	−
Rothia dentocariosa	ATCC 14190	A	Strong autoagglutination
R. dentocariosa	477	C	−
R. dentocariosa	842	C	−
R. dentocariosa	1003	C	−
Arachnia propionica	Golyadkin Jr.	A	−
A. propionica	Golyadkin Sr.	A	−
A. propionica	464	C	−
Lactobacillus casei	4961	A	−

[a] A, culture collection, University of Toronto, Faculty of Dentistry; B, E. D. Fillery, University of Toronto, Numerical Taxonomy Study (ref. 8); C, M. A. Gerencser, University of West Virginia.

[b] More rapid agglutination in mannose solution than in mannose-free control.

isolation. Bacterial cells for all experiments were grown for 2 days in a chemically defined medium for oral gram-positive rods (S. S. Socransky, C. Smith, and A. D. Manganiello, Int. Assoc. Dent. Res., abstr. no. 120, 1973). All cultures were incubated at 37°C in an atmosphere of 80% N_2, 10% H_2, and 10% CO_2 with a palladium catalyst.

Agglutination assays. Two standard assays for measuring the degree of *Actinomyces* agglutination were employed. The first, used for rapid screening, was a slide agglutination test conducted at room temperature. Bacterial cells were washed twice by centrifugation in 0.01 M phosphate-buffered saline at pH 6.0, dispersed by forceful pipetting, and resuspended in the same buffer to an optical density of 0.8 (Turner model 350 spectrophotometer). One drop of bacterial suspension was mixed with one drop of sugar solution yielding a final concentration of approximately 0.5 M. Aggregates were scored macroscopically 0 to 4+ according to the rapidity of aggregation observed compared with controls without sugars. The suspensions were also examined microscopically to detect aggregates.

The second assay compared the agglutination rates of sugar-containing and control suspensions by measuring their rates of decrease in optical density caused by the settling of bacterial aggregates to the bottom of test tubes (6, 15). Bacterial suspensions of 3 ml were prepared in the same buffer as those for the slide assay and incubated in a slowly shaking water bath at 37°C. The final concentration of the sugars for the tube assay was 0.14 M. The optical density was measured after 30, 60, 120, 180, and 240 min of incubation.

Solutions of the following sugars were tested in both the slide and tube assays for the ability to agglutinate *A. viscosus* and *A. naeslundii* (D-isomer unless mentioned): glucose, glucosamine, galactose, galactosamine, *n*-acetyl-galactosamine, fructose, L-fucose, talose, xylose, mannose, L-mannose, mannosamine, α-

methyl-mannoside, mannoheptulose and rhamnose (6-deoxy-L-mannose). The sugar alcohol mannitol and the polysaccharide yeast mannan were also tested. Analyzed grade chemicals were used whenever available. D-Mannose of various grades was purchased from several suppliers. These included Analyzed Biochemical and Ultrex grades (J. T. Baker Chemical Co., Phillipsburg, N.J.), biochemical grade (British Drug Houses, Ltd., Poole, England), mixed anomers (Sigma Chemical Co., St. Louis, Mo.), and unspecified grade (Difco). Protein concentrations of 1.0 M mannose solutions were determined by the method of Lowry et al. (12), using bovine serum albumin as a standard.

Experimental conditions affecting agglutination. By using the tube agglutination assay with a set of six fresh dental plaque isolates (designated 3-37fc, 3-40fc, 4-16a, 4-65a, 5-71b), we studied several experimental parameters affecting the agglutination reaction. Inocula for all experiments were taken from the same stock culture of each strain to avoid laboratory transfer. Mannose purchased from British Drug Houses was used for these experiments unless noted. The effect of heating the bacterial cells or the mannose solutions and the effect of running the tube assay at various temperatures was studied. The agglutination of bacterial cells treated by heating in a water bath at 60°C for 1 h was compared with that of control cells heated at 37°C for the same period. The ability of either boiled or autoclaved (15 lb [ca. 6.8 kg], 15 min) mannose solutions to agglutinate *Actinomyces* cells was compared with that of unheated control solutions. The rate of *Actinomyces* agglutination at different temperatures was tested by simultaneous incubation of matched bacteria-mannose solution suspensions at 37 or 4°C and ambient temperature. In addition, the ability of mannose solutions containing 25 μg of chloramphenicol per ml to agglutinate *Actinomyces* cells was compared with that of control mannose solutions without chloramphenicol to test the possibility that the bacteria could be synthesizing an agglutinin when suspended in the mannose solutions.

Sugar inhibition of mannose solution enhanced agglutination. By using both the slide and tube assays, we studied the relative ability of various sugars to competitively inhibit mannose solutions from agglutinating *Actinomyces* cells. The sugars which were purchased commercially included the following (D-isomer unless stated): glucose, glucosamine, *N*-acetyl-glucosamine, galactose, galactosamine, *N*-acetyl-galactosamine, lactose, L-fucose, *N*-acetyl-neuraminic acid, α-methyl-mannoside, melibiose, and rhamnose. The final concentration in the agglutination assay mixtures was 0.1 M. The sugar inhibition assays were tested for each of four fresh isolates (4-16a, 4-30a, 4-68a, 4-65a).

A. viscosus and *A. naeslundii* contain the unique cell wall sugar 6-DOT which is not available commercially. 6-DOT was isolated from *A. viscosus* strain P2 by a modification of the procedure of Tylenda et al. (21) and then tested for competitive inhibition of agglutination. Batch cultures of strain P2 were grown in brain heart infusion broth. Cells were harvested and washed by refrigerated centrifugation and lyophilized. A 0.6-g fraction of dried cells was obtained. The cells were hydrolyzed at a concentration of 1.25 mg/ml in 2 N HCl at 100°C for 2 h and lyophilized over KOH. The residue was dissolved in 10% isopropanol. The solution was applied in multiple spots to Whatman no. 3 chromatography paper. Sugars were separated by descending chromatography by using a pyridine-butanol-water solvent system at a ratio of 6:4:3. The chromatogram of the end spot was separated from the remainder, developed by the aniline-phthalate reaction, and used as a guide to locate the region occupied by rhamnose and the faster-moving 6-DOT on the unstained chromatogram. These regions were separated from the rest containing other carbohydrate-containing spots, rechromatographed in a direction perpendicular to the original, and eluted off the edge of the paper to isolate rhamnose and 6-DOT. The volatile solvents were allowed to evaporate, and the residues were lyophilized. The concentration of methyl pentose sugar in the reconstituted solutions was determined by the method of Dische and Shettles (3), using L-fucose as a standard. Portions of the two samples were rechromatographed to assure that either rhamnose or 6-DOT was the only carbohydrate present; commercially purchased rhamnose was used as a control. The remaining solution was mixed with the mannose and bacterial suspensions to determine their effects on aggregation in both the slide and tube assays.

Agglutination with D-mannose mother liquors. The mother liquors from which D-mannose is crystallized commercially were obtained from Pfanstiehl Laboratories, Inc. (Waukegan, Ill.). Water-soluble portions of the mother liquors were dissolved to yield a 10% (vol/vol) solution. This solution and two-fold serial dilutions were tested for *Actinomyces* agglutinating activity. The 10% aqueous mother liquor solution was fractionated by ascending gel filtration at ambient temperature on columns of Sephadex G-75 and G-50 (1 by 60 cm; Pharmacia [Canada] Ltd., Dorval, Quebec, Canada). Fractions were eluted with 0.1 M Tris-hydrochloride at pH 7.5 at a flow rate of 3 ml/h. Void volumes and column volumes were estimated by elution profiles for blue dextran and tyrosine, respectively. *Actinomyces* agglutinating activity of the fractions was tested by the slide assay. The protein concentration of the fractions was determined (12). The 10% mother liquor solution was also dialyzed against daily changes of 1 liter of water at 4°C for 5 days using a dialysis tubing (Spectrapor, Spectrum Medical Industries, Inc., Los Angeles, Calif.) with an exclusion pore size corresponding to a molecular weight of 3,500. A portion was removed from within the dialysis bag each day and tested for bacterial agglutination. In a second experiment, the mother liquor solution was dialyzed against an equal volume of water without daily changes. Samples were removed from inside and outside the tubing and used in the slide agglutination assay.

RESULTS

Agglutination specificity of D-mannose solutions. Most of the strains of *A. viscosus* and *A. naeslundii* which belonged to clusters containing human isolates by numerical taxonomy (clusters 1–6, reference 8) were agglutinated

by solutions of D-mannose. Among the 22 laboratory strains, 16 agglutinated (Table 1). A total of 77 of the 80 fresh isolates also agglutinated. Figure 1 illustrates typical tube agglutination reactions for two of the fresh isolates. Strain 4-16a agglutinated much more rapidly in solutions of D-mannose than in control solutions. Strain 4-71a was self-agglutinating, but even this strain agglutinated more rapidly with D-mannose solutions. Microscopic examination of the settled bacteria demonstrated large cohesive aggregates which could not be dispersed easily by agitation. None of the other sugars tested agglutinated *A. viscosus* or *A. naeslundii* strains. Mannitol and yeast mannan also lacked agglutinating activity. Agglutination by mannose solutions was observed for one of the three rodent-derived *A. viscosus* strains but not for laboratory strains of other gram-positive rods indigenous to the human mouth (Table 1). One strain of *Actinomyces odontolyticus* (ATCC 17982) aggregated with mannose but not with the control solution in the tube assay after 2 h of incubation.

A. viscosus and *A. naeslundii* agglutinating activity was detected in all the various grades of D-mannose purchased from the four chemical suppliers. Figure 2 compares the relative ability of the products to agglutinate *Actinomyces* cells by using the mean figure derived for all six fresh isolates tested at each time period. The J. T. Baker and Sigma Chemical Corp. products contained weaker agglutinating activity than the British Drug Houses and Difco products but still enhanced agglutination when compared with the mannose-free control suspensions. Each of the D-mannose products contained microgram quantities of protein per milliliter of 1.0 M solutions (Table 2). The protein concentration was highest for the best *Actinomyces* agglutinators, Difco and BDH (Fig. 2).

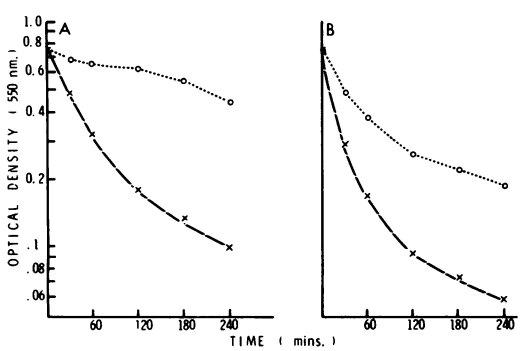

FIG. 1. *Examples of enhanced agglutination rates of Actinomyces cells by D-mannose solutions (×) when compared with mannose-free controls (○) using the tube agglutination assay. Curves are typical for both non-self-agglutinating strains (A, 4-16a) and self-agglutinating strains (B, 4-71a).*

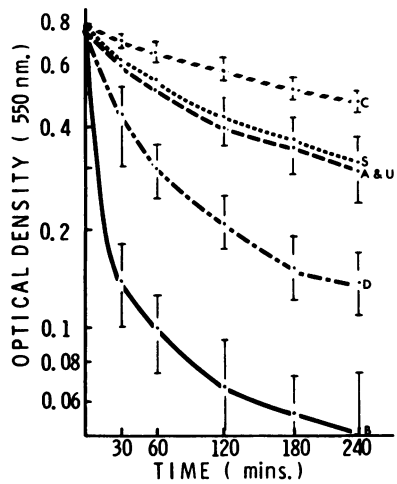

FIG. 2. *Mean (± standard error of mean) Actinomyces agglutination rates for six fresh isolates suspended in solutions of D-mannose purchased from various suppliers: (C) mannose-free control; (S) Sigma; (A) Baker analyzed biochemical; (U) Baker Ultrex; (D) Difco; and (B) British Drug Houses.*

TABLE 2. *Protein concentration in a 1.0 M solution of D-mannose*

Chemical supplier	Grade	Protein concn (μg/ml)
J. T. Baker	Ultrex	70
J. T. Baker	Analyzed	90
Sigma Chemical Corp.	Mixed anomers	107
British Drug Houses	Biochemical	135
Difco Laboratories	Unspecified	178

Experimental conditions affecting agglutination. Figure 3 illustrates mean data demonstrating the effects of varying the conditions of the tube agglutination assay on the agglutination of six fresh isolates of *A. viscosus* and *A. naeslundii*. Mannose solution-enhanced agglutination was affected little by heating the bacterial cells to 60°C for 1 h (Fig. 3B), by running the assay at either 37 or 4°C or at room temperature (Fig. 3C), or by incorporating chloramphenicol in the reaction mixture (Fig. 3D). The rate of agglutination was retarded by either boiling or autoclaving the mannose solutions (Fig. 3A). Although neither treatment reduced agglutination activity to control levels, autoclaving the mannose solution was more effective than boiling.

Sugar inhibition of agglutination. Hapten inhibition experiments using sugars purchased commercially failed to identify any compound

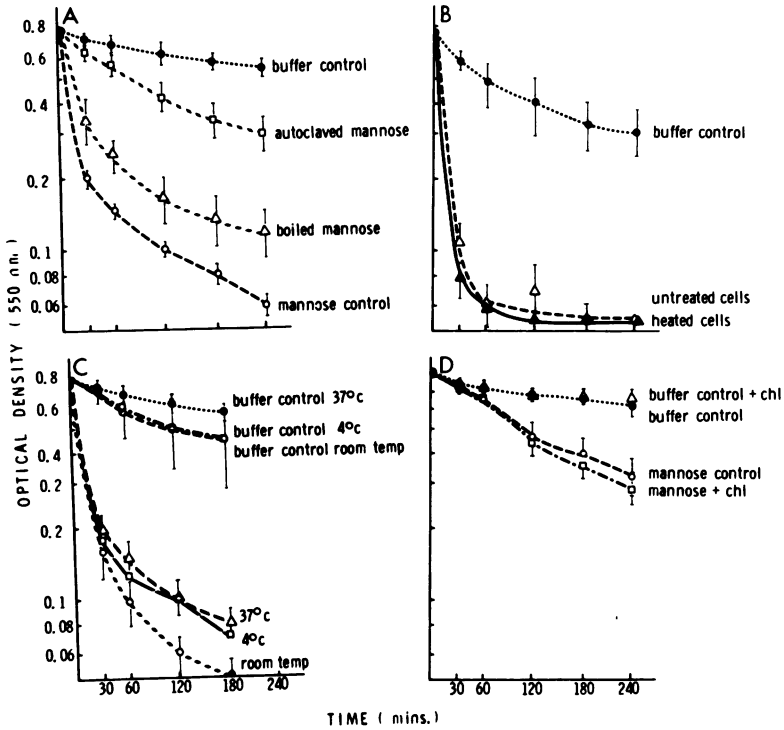

FIG. 3. *Mean (± standard error of mean) agglutination rates for six fresh isolates under various experimental conditions. (A–C)* D*-Mannose used for experiments was from British Drug Houses.* D*-Mannose used in chloramphenicol experiment (D) was J. T. Baker analyzed biochemical grade. (A) Boiling mannose solution. (B) Heating bacteria at −60°C for 1 h. (C) Assay temperature. (D) Addition of chloramphenicol (chl).*

which impaired mannose solution agglutination of *A. viscosus* and *A. naeslundii* at concentrations lower than 100 mM. Figure 4 illustrates mean data for four fresh isolates, demonstrating the dissimilar effects of rhamnose-containing and 6-DOT-containing extracts of *A. viscosus* strain P2 on their mannose-enhanced agglutination. The extract containing 6-DOT at an estimated concentration of 0.7 mM reduced agglutination of all strains to rates equivalent to their agglutination in the mannose-free buffer controls. Both strain P2 cell extracts containing rhamnose and commercially purchased rhamnose at a final concentration of 100 mM failed to impair mannose-enhanced agglutination.

Agglutination by D-mannose mother liquors. D-Mannose mother liquors contained water-soluble agglutinins for *A. viscosus* and *A. naeslundii* which could be diluted 16-fold before losing agglutinating activity for the strains tested (Table 3). Figure 5 compares the protein concentrations and *Actinomyces* agglutinating activity of Sephadex G-50 fractions of a nondialyzed 10% solution of mother liquors. The fractions with agglutinating activity corresponded reasonably

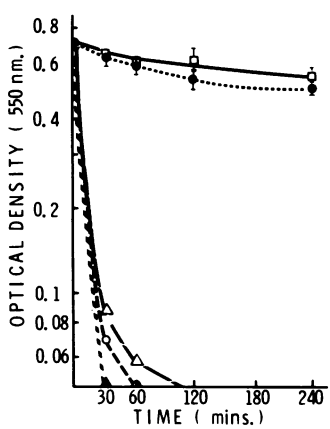

FIG. 4. *Mean (± standard error of mean) agglutination rates for four fresh isolates tested for inhibition by A. viscosus P2 extracts containing either 6-DOT or rhamnose (6-deoxy-*L*-mannose) at concentrations of 0.7 mM. Commercially purchased rhamnose was used at a final concentration of 100 mM. Symbols: □, mannose + extracted 6-DOT; ●, buffer control; ▲, mannose control; ○, mannose + extracted rhamnose; △, mannose + commercial rhamnose.*

well with the fractions containing a major protein peak. Maximum agglutinating activity eluted in fractions 41–44 which were found to be late within the column volume of G-50 columns. Fractions containing *Actinomyces* agglutinating activity eluted slightly beyond the estimated column volume when Sephadex G-75 was used. These data suggested that the agglutinins were of low molecular weight, probably below 4,000. This suggestion was supported by the dialysis experiments. Agglutinating activity of the mother liquor solution was lost after 1 day of dialysis against large volumes of water by using dialysis tubing with an exclusion pore size approximating 3,500 daltons. When dialyzed against a volume of water equal to that of the mother liquor solution, the agglutinating activities of samples taken from inside and outside the tubing were equivalent.

DISCUSSION

Although many strains of *Actinomyces* agglutinate slowly and spontaneously (6, 7), it is evident from the data presented that the agglutination of most strains of *A. viscosus* and *A. naeslundii* is enhanced greatly by solutions of D-mannose. A total of 73% of laboratory strains and 97% of fresh isolates agglutinated rapidly. The strains agglutinated in solutions of mannose purchased from several suppliers but not in solutions of other monosaccharides. These and other sugars also failed to act as competitive inhibitors of agglutination when incorporated into mannose-containing bacterial suspensions. Only 6-DOT-containing extracts of *A. viscosus* cells impaired aggregation at very low concentrations. Mannose-enhanced agglutination was also partially impaired by boiling or autoclaving the mannose solutions. Heating the bacteria, running the assay at various temperatures, or incorporating chloramphenicol in the reaction mixture failed to affect agglutination. These findings led to the hypothesis that commercially available D-mannose contains a relatively heat-labile contaminating agglutinin for *A. viscosus* and *A. naeslundii* with a possible selective cell wall sugar affinity. Our finding that mother liquors, which are by-products of the manufacture of D-mannose, also contain protein-associated *Actinomyces* agglutinins lends support to this contention.

The finding that commercial sources of monosaccharides contain agglutinins is not unique to this study. Marquardt and Gordon reported that several monosaccharide products, including D-mannose, contain hemagglutinins, and that the degree of their hemagglutinating activity depended on the commercial source (13). The range of products with *A. viscosus* and *A. naeslundii* agglutinating activity is much narrower,

TABLE 3. *Agglutination of Actinomyces cells with 10% mother liquor solutions*

Bacterial strain	Agglutination[a] with:		Dilution factor				
	Water	10% mother liquor[b]	2	4	8	16	32
38fc	0	4+	4+[a]	4+	3+	1+	0
W752	0	4+	4+	4+	4+	1+	±
H21	1+	4+	4+	4+	4+	3+	1+
WVU627	0	4+	4+	4+	3+	2+	±
P2	±	4+	ND[c]				
N9	0	3+	ND				
C2	1+	4+	ND				
B120	0	3+	ND				
11B2	0	3+	ND				
4A05	±	4+	ND				

[a] 0, None detectable; 4+, maximum agglutination.
[b] 10% (vol/vol) aqueous solution.
[c] ND, Not done.

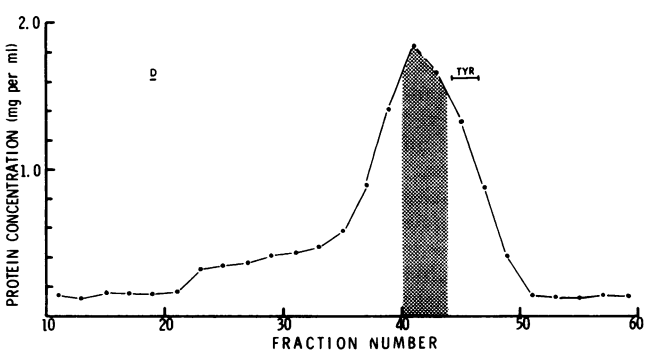

FIG. 5. *Comparison of Actinomyces agglutinating activity and protein concentration of fractions of aqueous D-mannose mother liquor solution separated by gel filtration on Sephadex G-50. Shaded area represents fractions with agglutinating activity. Elution positions for blue dextran (D) and tyrosine (TYR) estimate the void and column volumes.*

being limited solely to D-mannose. Even compounds with chemical structures approximating that of D-mannose (mannosamine, mannoheptulose, α-methyl-mannoside, mannitol, and yeast mannan) failed to agglutinate *Actinomyces* cells. Similar to the hemagglutinins found in monosaccharides, various grades of D-mannose purchased from several suppliers differed in their *Actinomyces* agglutinating activities. D-Mannose products contaminated with higher concentrations of protein-like material generally agglutinated the bacteria most rapidly. An exact correlation of protein content and agglutinating activity was not noted, probably due to protein contaminants other than the specific agglutinin. However, even Ultrex grade mannose (J. T. Baker), which is reputed to be of highest quality, was found to contain detectable protein and have considerable agglutinating activity for some *Actinomyces* strains.

Among the bacterial strains tested, rapidly enhanced agglutination was observed only for strains designated *A. viscosus* and *A. naeslundii*. These findings suggest that mannose solution agglutination may find future use as an adjunct in the identification of clinical isolates or as a research tool in studying inhibitors of selective attachment to oral surfaces. Only gram-positive rods predominant in the oral cavity were tested because of the difficulties associated with rapidly differentiating this group. It is possible that the apparent selectivity of the mannose-associated agglutinins for *A. viscosus* and *A. naeslundii* demonstrated in this study will not extend beyond the species tested.

Data from experiments examining various parameters affecting mannose-enhanced agglutination suggest that the activity relates to a contaminant and not to utilization of the mannose itself either as the agglutinin or as a substrate for the rapid synthesis of an agglutinin. The agglutinating activity of mannose solutions was partially sensitive to heat by boiling or autoclaving. Autoclaving resulted in greater inhibition, probably because of more prolonged exposure to heat during the slow exhaust cycle. Sensitivity to heating would be more consistent if the agglutinin were protein rather than carbohydrate in nature. In contrast, agglutinating ability of the *Actinomyces* cells was not affected by heating at 60°C for 1 h. The findings that agglutination proceeded with equal rapidity in the cold as at 37°C and that incorporation of chloramphenicol in the assay mixture had no effect suggest that the bacteria do not use the contents of the mannose solutions for the rapid synthesis of an agglutinin.

The selectivity of the heat-sensitive, protein-associated agglutinin for a component of the *Actinomyces* cell surface raises the possibility that the agglutinin may function by a "lectin-like" mechanism. The derivation of D-mannose from the seed (vegetable ivory nut) of the Tagua palm, *Phytelephas macrocarpa*, supports this possibility. Demonstration of specific sugar binding activity, usually by competitive hapten inhibition assays, is essential before considering an agglutinin to be a lectin (19). None of the common monosaccharides tested interfered with mannose-enhanced agglutination, even at concentrations as high as 100 mM. However, a major sugar component of *A. viscosus* and *A. naeslundii* cell walls, 6-deoxy-L-talose, is not available from commercial sources and had to be extracted from a recognized *A. viscosus* strain. The extract containing approximately 0.7 mM 6-DOT and no other detectable sugar totally inhibited agglutination of the four strains tested. Although not conclusive, these findings suggest the hypothesis that the mannose-contaminating agglutinin may function like a lectin with an affinity for a sugar limited to *A. viscosus*, *A. naeslundii*, and *A. odontolyticus* strains among oral gram-positive rods (1, 9). If this were so, the failure of some known 6-DOT-containing laboratory strains of *A. viscosus* and *A. naeslundii* to agglutinate could have been caused by the inaccessibility of their 6-DOT. A similar masking of attachment-related receptors by a copious capsular material has been proposed to impair colonization of an *A. viscosus* mutant strain, T14AV (2). It is interesting to note that *A. viscosus* and *A. naeslundii* strains themselves possess surface-associated components with lectin-like affinity for specific strains of *Streptococcus sanguis* (14) and for erythrocytes (R. P. Ellen, E. D. Fillery, K. H. Chan, and D. A. Grove, Int. Assoc. Dent. Res., Abstr. no. 999, 1979). These reactions are inhibited specifically by low concentrations of β-galactosides, which are totally ineffective in blocking the mannose-contaminating agglutinin.

The mother liquors from which Pfanstiehl Laboratories crystallizes D-mannose also contained a water-soluble agglutinin for *A. viscosus* and *A. naeslundii*. The agglutinating activity could be isolated by column chromatography in fractions containing a major protein component. The agglutinin has been estimated to be of low molecular weight by its late elution from Sephadex G-50 columns and its dialysis through tubing of small pore size. Should the *Actinomyces* agglutinin be of diagnostic or research value in the future, the mother liquors or the seeds themselves would probably be a better source than the "purified" mannose.

ACKNOWLEDGMENTS

This investigation was supported by grant MA 5619 from the Medical Research Council of Canada and Public Health Service grant DE 04464 from the National Institute of Dental Research.

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