EPIDEMIOLOGY OF INFECTION

Lectin–oral streptococci interactions

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Lectins of various specificities were examined for interaction with strains of oral streptococci of various species. The lectins were capable of binding galactose, *N*-acetylgalactosamine, glucose, *N*-acetylglucosamine, mannose, fucose and sialic acid. Lectin reactivities were strain-dependent in that some members of a species, but not others, could be aggregated by certain lectins. Proteolysis and extraction with hot water, guanidine hydrochloride and sodium dodecyl sulphate tended to increase the reactivity of the streptococci with lectins but did not change the recognition patterns of the bacteria. Sonication, in contrast, tended to reduce the ability of streptococci to be agglutinated by lectins. Furthermore, lectin reactivities were dependent on the growth conditions, as evidenced by changes in lectin titres following streptococcal growth in sub-inhibitory concentrations of citrate, fluoride or antibiotics. It is likely that lectins could be useful tools for epidemiological studies and to probe strain-dependent and growth-dependent surface characteristics of viridans streptococci.

Introduction

In microbiology, lectins have assumed an important role in studying glycoconjugates and microbial cell surfaces. In bacteria, there are several glycoconjugates capable of serving as receptors for lectins. These include peptidoglycans (PG), teichoic acids, lipoteichoic acids, capsules, surface arrays, glycoproteins (unusual in most prokaryotes), glycolipids, lipopolysaccharides and secreted polysaccharides. In theory, any glycoconjugate may act as a receptor, provided that a lectin of the proper specificity can be identified.

One particular use of lectins in microbiology has been in laboratory diagnostic microbiology [1–5]. Thus, the binding of a particular isolate by a particular lectin may be used to identify the organism. There are numerous reports of the uses of lectins in diagnostic microbiology. A gram-negative diplococcus growing on Thayer-Martin medium and capable of being aggregated by 5–10 µg of wheat-germ agglutinin (WGA) is either *Neisseria gonorrhoeae* or untypable *N. meningitidis* [6]. The latter organism is quickly identified by the presence of γ glutamyl-aminopeptidase [5]. Also, *Campylobacter* spp., for example, can

be distinguished by lectins [7]. Not only are lectins important as diagnostic probes, the proteins are useful in epidemiological studies, in which lectin reactivity is dependent on the geographic source of the isolate [8, 9].

The aim of this research was to determine if lectins could be employed as reagents for oral streptococci. It is already known that some streptococci possess receptors for lectins. Other research has shown that streptococcal polymers, such as lipoteichoic acids from *Enterococcus faecalis* and *E. hirae*, bind avidly to concanavalin A (Con A)-agarose columns, the avidity of which is a function of polymer glucosylation [10]. In group A streptococci, Con A coupled with ferritin has been used as a probe for lipoteichoic acid on the cell surface [11]. In group C streptococci, the lectin from *Helix pomatia* (HPA) offers a selective reagent for cell agglutination [12, 13]. Wheat-germ agglutinin (WGA, see Table 1 for abbreviations) offers a convenient probe for various streptococcal polymers [14]. Kashket and Guilmette [15] observed that several oral streptococci could be readily aggregated by Con A, but dextranase treatment of the bacteria reduced the interaction with the lectin. Studies by Hamada *et al.* [16] revealed that lectins capable of binding to oral streptococci could reduce the adhesion of the bacteria to saliva-coated hydroxylapatite.

This study was performed to discover if a particular lectin, or group of lectins, can agglutinate particular

oral streptococci. This knowledge may be useful in studying the distribution of the bacteria in the oral cavity, and lectins may prove to be of use in the study of the epidemiology of caries in various populations.

Materials and methods

Bacterial strains

A collection of 20 strains of 10 species of oral streptococci and one strain of *E. faecalis* was used for this study. Bacteria were obtained from the American Type Culture Collection or from Professor A. Bleiweis, University of Florida (USA). Stock cultures were maintained in Tryptic Soy Broth (TSB; Sigma) at -70°C and were streaked on Mitis-Salivarius (MS) agar (Difco Laboratories) to obtain isolated colonies. Gram-staining and the catalase test were performed routinely to identify organisms presumptively as streptococci. Classical biochemical tests were used to confirm the identity of the bacteria [17–19]. The streptococci were cultured in TSB; before autoclaving, the broth was incubated with fungal dextranase (Sigma; 0.65 units of enzyme preparation/g of dry medium) at 37°C for 2 h and with yeast invertase (0.2 mg/g of dry

medium) at 55°C for 2 h to remove any sucrose or dextran, known to promote autoagglutination [20].

Culture (growth) conditions

Isolated colonies of oral streptococci from MS agar plates were subcultured in TSB, incubated statically at 37°C in an aerobic atmosphere supplemented with CO_2 5% overnight for *c.* 18 h to late exponential phase. The bacteria were then harvested by centrifugation (Sorvall RC-5B refrigerated superspeed centrifuge, Newtown, CT, USA) at 10 000 *g* for 5 min and washed twice with phosphate-buffered saline (PBS; 20 mM sodium phosphate 150 mM sodium chloride, pH 7.3). Bacterial cell pellets were then suspended in PBS to an appropriate optical density, judged by absorbance at 540 nm (Milton Roy Spectronic 21D, Rochester, USA).

Plant lectins

The lectins used were selected on the basis of their reported specificities to cover the widest possible range of carbohydrates (Table 1). All lectins were commercially available from E-Y Laboratory (San Mateo, CA, USA) and Lectinola (Charles University, Prague, Czech

Table 1. Panel of lectins*

Systematic name	Abbreviation and common name	Sugar specificities
<i>Abrus precatorius</i>	APA (jequirty bean)	β -D-Gal > α -D-GalNAc
<i>Agaricus bisporus</i>	ABA (mushroom)	Gal β -1,3GalNAc; Gal
<i>Anguilla anguilla</i>	AAA (fresh water eel)	L-Fuca-1,3Gal[Anti-O(H)]
<i>Arachis hypogaea</i>	PNA (peanut)	Gal β -1,3-GalNAc; Gal (Anti-T ₁ T _k T _h T _x)
<i>Artocarpus integrifolia</i>	AIA (jackfruit)	Gal β -1,3GalNAc (Anti-T)
<i>Bauhinia purpurea</i>	BPA (camel's foot tree)	Gal β -1,3GalNAc; Gal β -1,3Gal (Anti-T)
<i>Canavalia ensiformis</i>	Con A (jack bean)	Mana-1; Glca-1; GlcNAc α -1
<i>Datura stramonium</i>	DSA (jimson weed)	Gal β -1,3 (4) GlcNAc
<i>Dolichos biflorus</i>	DBA (horse gram)	GalNAc α -1,3 GalNAc (Anti-A ₁ > A ₂ > Cad)
<i>Erythrina cristagalli</i>	ECA (coral tree)	Gal β -1,4GlcNAc; Gal α 1,6Gal; Gal
<i>Euonymus europaeus</i>	EEA (spindle tree)	Complex
<i>Galanthus nivalis</i>	GNA (snowdrop bulb)	D-Mannose
<i>Glycine max</i>	SBA (soybean)	GalNAc α (or β)1,3Gal; D-Gal
<i>Griffonia simplicifolia I</i>	GSA-I (African legume)	α -D-Gal; D-GalNAc (Anti A, B)
<i>Griffonia simplicifolia II</i>	GSA-II (African legume)	D-GlcNAc (Anti-B, T _k T)
<i>Helix aspersa</i>	HAA (garden snail)	α -D-GalNAc (Anti-A)
<i>Helix pomatia</i>	HPA (edible snail)	α -D-GalNAc α -1,3GalNAc
<i>Laburnum alpinum</i>	LAA (Scotch laburnum)	L-Fuca-1,2Gal[Anti-O(H)]
<i>Lens culinaris</i>	LCH (lentil)	Mana-1; Glca-1
<i>Limax flavus</i>	LFA (garden slug)	Sialic acid
<i>Limulus polyphemus</i>	LPA (horseshoe crab)	Sialic acid
<i>Lycopersicon esculentum</i>	LEA (tomato)	(GlcNAc β -1,4) _n
<i>Maackia amurensis</i>	MAA	Sialic acid
<i>Maclura pomifera</i>	MPA (osage orange)	Gal β -1,3GalNAc (Anti-T)
<i>Misgurnus anguillicaudatus</i>	Loach	L-Rhamnose
<i>Phaseolus lunatus</i>	LBA (lima bean)	α -D-GalNAc α -1,3Gal
<i>Phaseolus vulgaris</i>	PHA-E (red kidney bean)	Complex
<i>Phytolacca americana</i>	PWA (pokeweed)	Gal β -1,4 (3) GlcNAc; (GlcNAc β -1,4) _n ; Mana-1,2Man
<i>Pisum sativum</i>	PEA (garden pea)	Mana-1; Glca-1
<i>Ricinus communis</i>	RCA-I, II (castor bean)	Gal β -1,3 (4) GlcNAc; Gal β -1,3 GalNAc; GalNAc
<i>Robinia pseudoacacia</i>	RPA (black locust)	Complex
<i>Solanum tuberosum</i>	STA (potato)	(GlcNAc β -1,4) ₂₋₅
<i>Sophora japonica</i>	SJA (pagoda tree)	Gal β -1,3GalNAc; Gal β -1,3GlcNAc
<i>Triticum vulgaris</i>	WGA (wheat-germ)	(GlcNAc β -1,4) _n ; sialic acid
<i>Ulex europaeus I</i>	UEA I (gorse, furze)	L-Fuca-1,2Gal[Anti-O(H)]
<i>Ulex europaeus II</i>	UEA II (gorse, furze)	(GlcNAc β -1,4) ₂ [Anti-O(H)]
<i>Vicia fava</i>	VFA (fava bean)	Mana-1; Glca-1
<i>Wisteria floribunda</i>	WFA (Japanese wisteria)	GalNAc α -1,6Gal; GalNAc; Gal

*Adapted from references 2 and 4.

Republic), except concanavalin A and *Lycopersicon esculentum* which were from Sigma. Powdered lectins were dissolved in PBS (pH 7.3) to a concentration of 1 mg/ml and stored at -20°C .

Agglutination assays

The streptococcal strains were grown and harvested, then suspended and adjusted to an OD_{540} of 1.0. Assays were performed in 'U'-bottom, 96-well disposable polystyrene microtitration plates (Dynatech Laboratories Inc., Alexandria, VA, USA) with 50 μl of lectin (original concentration 1 mg/ml), and 50 μl of bacterial suspension (OD_{540} 1.0). Negative controls were prepared by adding 50 μl of PBS instead of lectins to the bacterial suspensions. After gentle shaking for 15 min at room temperature and overnight incubation at 4°C , results were read on a test reading mirror (Dynatech Laboratories) by the settling pattern of bacteria and the presence of agglutinated cells.

Procedures for cell extraction or surface modification

Because lectin aggregation patterns may be altered by treatment of bacteria with proteases or chaotropes, or both, the streptococci were subjected to conditions that may result in cell surface modification. *S. sobrinus* and *S. cricetus* possess GBL, whereas *S. mutans* and *S. sanguis* are GBL-deficient. The washed bacteria in PBS were incubated with Pronase or trypsin 100 $\mu\text{g}/\text{ml}$ for 2 h at 37°C . The cells were also extracted with 6 M guanidine hydrochloride for 2 h at 21°C . Similarly, cells in water were extracted with SDS 100 $\mu\text{g}/\text{ml}$ for 2 h at 37°C . Cell suspensions were also heated in PBS at 100°C for 10 min or subjected to sonication for 1 min at 21°C .

Following each treatment the cells were washed twice with water, except for the SDS-treated cells which were washed five times to remove the detergent. The cells were finally suspended in PBS before mixing with lectins.

Sugar inhibition assays

To test whether the agglutination reaction of the lectin was caused by its carbohydrate-binding capacity, inhibition of agglutination was tested with several sugars. Equal volumes (25 μl) of carbohydrate, lectin and bacterial suspension were mixed to give a final inhibitor concentration of 50 mM. In the positive control, PBS replaced carbohydrate and the negative control consisted of PBS (50 μl) and bacterial suspension (25 μl).

Influence of subinhibitory concentrations (SICs) of fluoride, citrate or antibiotics on lectin titres

A subgroup of the streptococci (*S. cricetus* AHT, *S.*

sobrinus 6715, *S. mutans* 10449 and *S. sanguis* 10556) were cultured in TSB supplemented with 5 mM sodium fluoride, 20 mM sodium citrate, oxacillin 0.05 mg/L or streptomycin 10 mg/L (chemicals and antibiotics were from Sigma). Sodium fluoride and sodium citrate were dissolved in TSB before autoclaving. The antibiotics were diluted with PBS and sterilised by filtration through 0.22 μm pore-size filters (Millipore Corporation, Bedford, MA, USA) then added to the autoclaved culture medium. Streptococcal cultures reached exponential phase in the presence of subinhibitory concentrations of antibiotics normally at about 2 days. Agglutination assays were performed with Con A, HPA and WGA. Cells grown in TSB without inhibitors were used as control.

Rates of lectin-mediated aggregation

Rates of lectin-mediated aggregation were investigated with a subset of the streptococcal strains (*S. sanguis* 10556, *S. mutans* 10449, *S. cricetus* AHT and *S. sobrinus* 6715) and lectins (Con A, WGA and HPA) previously tested by end-point analyses in the main study. Suspensions of streptococci in PBS were mixed rapidly with lectin solutions as described previously. Absorbance readings were then made as a function of time [21, 22].

Results

The results of the aggregation assays are recorded in Table 2. None of the strains was aggregated with *Galanthus nivalis* (GNA) lectin, specific for mannose. The streptococci are not known to possess mannose-containing surface polysaccharides, so the results with GNA were predictable. In contrast, Con A, LCH and PEA, which are specific for glucose and mannose, aggregated all the streptococci; another glucose-binding lectin, VFA, failed to agglutinate any of the test strains. Agglutination patterns varied among strains of the same species; e.g. only some strains of *S. mutans* were agglutinated by GSA-II, and similar results occurred with some strains of *S. cricetus* and LEA.

When streptococci were subjected to various treatments before mixing with lectins, titres increased in some instances, but with no treatment except for sonication did the lectin titre significantly decrease (Table 3). For example, the lowest concentration of Con A needed to cause agglutination of *S. cricetus* AHT was 15.6 $\mu\text{g}/\text{ml}$. Following treatment with trypsin, the required amount of Con A was 3.91 $\mu\text{g}/\text{ml}$ and, after Pronase digestion, this was reduced to 0.98 μg . *S. mutans*, *S. sanguis* and *S. sobrinus* all behaved similarly following proteolysis. Sonication, in contrast, rendered the bacteria much less reactive with lectins. For *S. mutans* 10449, the amount of Con A needed for aggregation after sonication was 500 $\mu\text{g}/\text{ml}$. This is a remarkably high concentration considering that only

Table 2. Survey of lectin-promoted aggregation of oral streptococci*

Streptococcal strain	Agglutination with														
	D-Glucose/mannose-binding lectins							N-Acetyl-D-glucosamine-binding lectins							
	Con A	GNA	LCH	PEA	VFA	DSA	GSA-II	LAA	LEA	PHA-E	PWA	STA	UEA-II	WGA	
<i>S. mutans</i> 10449 [†]	+	-	+	+	-	-	+	-	+	-	-	-	-	+	
<i>S. mutans</i> 318-11	+	-	+	+	-	-	+	-	-	-	-	-	-	+	
<i>S. mutans</i> 330-T10	+	-	+	+	-	-	+	-	-	-	-	-	-	-	
<i>S. mutans</i> Ingbritt 162	+	-	+	+	-	-	+	-	+	-	-	-	-	-	
<i>S. mutans</i> NG-8	+	-	+	+	-	-	+	-	+	-	-	-	-	-	
<i>S. mutans</i> OMZ175	+	-	+	+	-	-	+	-	+	-	-	-	-	+	
<i>S. ericetus</i> AHT	+	-	+	+	-	-	+	-	-	-	-	-	-	+	
<i>S. ericetus</i> E49	+	-	+	+	-	-	+	-	+	-	-	-	-	+	
<i>S. ericetus</i> HS1	+	-	+	+	-	-	+	-	-	-	-	-	-	-	
<i>S. ericetus</i> HS6	+	-	+	+	-	-	+	-	-	-	-	-	-	-	
<i>S. ericetus</i> OMZ61	+	-	+	+	-	-	+	-	+	-	-	-	-	+	
<i>S. ericetus</i> 6715 [†]	+	-	+	+	-	-	+	-	+	-	-	-	-	+	
<i>S. sobrinus</i> B13	+	-	+	+	-	-	+	-	-	-	-	-	-	-	
<i>S. sanguis</i> 10556 [†]	+	-	+	+	-	-	+	-	-	-	-	-	-	+	
<i>S. gordonii</i>	+	-	+	+	-	-	+	-	-	-	-	-	-	+	
<i>S. mitior</i> 351-3	+	-	+	+	-	-	+	-	-	-	-	-	-	+	
<i>S. oralis</i>	+	-	+	+	-	-	+	-	-	-	-	-	-	+	
<i>S. anginosus</i> 27335 [†]	+	-	+	+	-	-	+	-	+	-	-	-	-	-	
<i>S. milleri</i>	+	-	+	+	-	-	+	-	+	-	-	-	-	-	
<i>S. salivarius</i> 13419	+	-	+	+	-	-	+	-	-	-	-	-	-	-	
<i>E. faecalis</i>	+	-	+	+	-	-	-	-	+	-	-	-	-	-	
N-Acetyl-D-galactosamine-binding lectins															
Streptococcal strain	D-Galactose-binding lectins							N-Acetyl-D-glucosamine-binding lectins							
	APA	ECA	GSA-I	MPA	PNA	RCA-I	RCA-II	BPA	DPA	HAA	HPA	LBA	SBA	STA	WFA
	+	+	-	-	+	+	+	+	+	-	+	-	-	-	+
<i>S. mutans</i> 10449 [†]	+	+	-	-	+	+	+	+	-	-	+	-	-	-	+
<i>S. mutans</i> 318-11	+	-	-	-	+	+	-	-	-	-	-	-	-	-	+
<i>S. mutans</i> 330-T10	+	-	-	-	+	+	+	-	-	-	-	-	-	-	+
<i>S. mutans</i> Ingbritt 162	+	+	-	-	+	+	+	-	-	-	+	-	-	-	+
<i>S. mutans</i> NG-8	+	-	-	-	-	+	+	-	-	-	-	-	-	-	+
<i>S. mutans</i> OMZ175	+	+	+	+	+	+	+	+	-	-	+	-	-	-	+
<i>S. ericetus</i> AHT	+	+	+	-	+	+	+	-	-	-	+	-	-	-	+
<i>S. ericetus</i> E49	+	+	+	-	+	+	+	-	-	-	+	-	-	-	+
<i>S. ericetus</i> HS1	+	-	+	-	+	+	+	-	-	-	-	-	-	-	+
<i>S. ericetus</i> HS6	+	-	+	-	+	+	+	-	-	-	-	-	-	-	+
<i>S. ericetus</i> OMZ61	+	+	+	+	+	+	+	+	-	-	+	-	-	-	+
<i>S. sobrinus</i> 6715 [†]	+	+	+	+	+	+	+	+	-	-	+	-	-	-	+
<i>S. sobrinus</i> B13	+	-	-	-	+	+	+	+	-	-	-	-	-	-	+
<i>S. sanguis</i> 10556 [†]	+	-	+	-	+	+	+	+	-	-	+	-	-	-	+
<i>S. gordonii</i>	+	-	+	-	+	+	+	+	-	-	+	-	-	-	+
<i>S. mitior</i> 351-3	+	-	+	-	+	+	+	+	-	-	+	-	-	-	+
<i>S. oralis</i>	+	-	+	-	+	+	+	+	-	-	-	-	-	-	+
<i>S. anginosus</i> 27335 [†]	+	-	-	-	+	+	+	-	-	-	-	-	-	-	+
<i>S. milleri</i>	+	-	-	-	+	+	+	-	-	-	-	-	-	-	+
<i>S. salivarius</i> 13419	+	-	-	-	+	+	+	-	-	-	-	-	-	-	+
<i>E. faecalis</i>	+	-	-	-	+	+	+	+	-	-	-	-	-	-	+

Streptococci	Fucose-binding lectins			Sialic acid-binding lectins			Rhamnose-binding lectin	Lectins with complex binding sites				
	AAA	Lotus	UEA-I	LFA	LPA	MAA		ABA	AIA	EEA	PHA-M	RPA
<i>S. mutans</i> 10449 [†]	-	-	-	+	+	-	+	-	+	-	-	+
<i>S. mutans</i> 318-11	-	-	-	+	ND	-	+	-	+	-	-	+
<i>S. mutans</i> 330-T10	-	-	-	-	ND	-	+	-	+	-	-	-
<i>S. mutans</i> Ingbritt 162	-	-	-	-	ND	-	-	-	+	-	-	+
<i>S. mutans</i> NG-8	-	-	-	-	ND	-	-	-	+	-	-	-
<i>S. mutans</i> OMZ175	-	-	-	-	+	-	-	+	+	-	-	-
<i>S. cricetus</i> AHT	-	-	-	+	+	-	-	-	+	-	-	+
<i>S. cricetus</i> E49	+	+	+	+	ND	-	+	-	+	-	-	+
<i>S. cricetus</i> HS1	-	-	-	+	ND	-	+	-	+	-	-	+
<i>S. cricetus</i> HS6	-	-	-	+	ND	-	+	-	+	-	-	+
<i>S. cricetus</i> OMZ61	+	+	+	+	ND	-	+	-	+	-	-	+
<i>S. sobrinus</i> 6715 [†]	-	+	+	+	+	-	+	+	+	-	-	+
<i>S. sobrinus</i> B13	-	-	-	-	-	-	+	+	+	-	-	-
<i>S. sanguis</i> 10556 [†]	-	-	-	+	+	-	+	+	+	-	-	+
<i>S. gordonii</i>	-	-	-	+	+	-	+	+	+	-	-	-
<i>S. mitior</i> 351-3	-	-	-	+	-	-	+	+	+	-	-	+
<i>S. oralis</i>	-	-	-	-	-	-	-	+	+	-	-	-
<i>S. anginosus</i> 27335 [†]	-	-	-	-	-	-	-	-	+	-	-	-
<i>S. milleri</i>	-	-	-	-	-	-	-	-	+	-	-	-
<i>S. salivarius</i> 13419 [†]	-	-	-	+	+	-	-	-	+	-	-	-
<i>E. faecalis</i>	+	+	+	-	-	-	-	+	+	-	-	-

* Aggregation involved the mixing of equal amounts of lectin solution (1.0 mg/ml) with washed cell suspensions. Aggregations were done on Boerner plates (readings made within 20–30 min) and plastic microwell plates (readings made after overnight incubations at 4°C). ND, not determined.

[†] ATCC strains.

Table 3. Aggregation of oral streptococci after various treatments

Streptococcal strain	Treatment	Minimum amount of lectin required for aggregation ($\mu\text{g/ml}$)			
		Con A	HPA	PNA	WGA
<i>S. cricetus</i> AHT	None	15.6	3.91	62.5	62.5
	Guanidine HCl	3.91	1.95	31.3	15.6
	Heat	7.81	3.91	62.5	31.3
	Protease	0.98	0.98	31.3	7.81
	SDS	1.95	1.95	15.6	15.6
	Sonication	125	31.5	ND	ND
	Trypsin	3.91	0.98	31.3	7.81
<i>S. mutans</i> 10449	None	15.6	62.5	62.5	250
	Guanidine HCl	3.91	15.6	31.3	31.3
	Heat	15.6	15.6	62.5	125
	Protease	1.95	1.95	31.3	7.81
	SDS	7.81	7.81	62.5	15.6
	Sonication	500	ND	ND	ND
	Trypsin	1.95	3.91	7.81	7.81
<i>S. sanguis</i> 10556	None	15.6	7.81	125	15.6
	Guanidine HCl	3.91	1.95	31.3	1.98
	Heat	7.81	7.81	125	15.6
	Protease	1.95	1.95	7.81	1.95
	SDS	1.95	0.98	31.3	3.91
	Sonication	125	250	ND	500
	Trypsin	1.95	1.95	15.6	3.91
<i>S. sobrinus</i> 6715	None	15.6	7.81	250	62.5
	Guanidine HCl	7.81	3.91	125	15.6
	Heat	7.81	7.81	250	31.3
	Protease	1.95	0.98	31.3	3.91
	SDS	1.95	3.91	31.3	15.6
	Sonication	125	250	ND	ND
	Trypsin	1.95	1.95	31.3	7.81

ND, not detected.

15.6 $\mu\text{g/ml}$ of the lectin was needed to elicit aggregation of the control cells.

Some streptococci produce surface-located glucan-binding lectins (GBLs). These bacteria, which include *S. cricetus* and *S. sobrinus* [20], are able to bind 6–10 α -1,6 linked glucose residues in their active sites. When the sites are filled, the bacteria are less hydrophobic, suggesting that the α -1,6 glucans had covered the streptococcal surfaces [23]. With this in mind, several streptococci, including *S. mutans* and *S. sanguis*, which do not possess GBLs, were mixed with glucan T-8 100 $\mu\text{g/ml}$ and then with HPA and WGA. The results, presented in Table 4, show that glucan did not alter the titres of either HPA or WGA with the cells (a difference of at least two dilutions are needed for a result to be considered significant).

When various oral streptococci were grown in medium containing citrate, fluoride, oxacillin or streptomycin,

it was found that lectin reactivities were altered (Table 5). Citrate caused a significant decrease in the amount of Con A needed to aggregate the bacteria. Similar results were obtained for HPA and WGA. Streptomycin, on the other hand, increased the amount of lectin (except for WGA and *S. sobrinus* 6715) needed to effect cell agglutination. Oxacillin lowered the amounts of lectins needed for aggregation of all bacteria tested. Based on these results, it would appear that growth conditions alter lectin reactivities.

Plots of absorbance–time relationships [21, 22] for selected streptococcal strains in the presence of Con A, WGA and HPA, are shown in Figs. 1–3. Both HPA and WGA rapidly aggregated *S. sanguis* 10556, but neither lectin was able to rapidly aggregate *S. mutans* 10449. The latter observation was interesting in view of the fact that *S. mutans* 10449 was reactive with HPA and WGA when analysed by end-point aggregations.

Table 4. Influence of α -1,6 glucan on lectin binding by oral streptococci*

Streptococcal strain	Minimum amount of lectin required for aggregation ($\mu\text{g/ml}$)			
	HPA	HPA + glucan	WGA	WGA + glucan
<i>S. cricetus</i> AHT	3.91	3.91	62.5	31.5
<i>S. mutans</i> 10449	62.5	125	250	250
<i>S. sanguis</i> 10556	7.81	7.81	15.6	31.5
<i>S. sobrinus</i> 6715	7.81	7.81	62.5	62.5

*Glucan T-8 was employed at a final concentration of 100 $\mu\text{g/ml}$.

Table 5. Agglutination of oral streptococci by lectins after growth of the bacteria in media with antibiotics, citrate or fluoride*

Streptococcal strain	Medium additive	Minimum amount of lectin (μg) required for aggregation		
		Con A	HPA	WGA
<i>S. cricetus</i> AHT	None	15.6	3.91	62.5
	Citrate	3.91	0.98	15.6
	Fluoride	15.6	15.6	62.5
	Oxacillin	7.81	1.95	3.91
	Streptomycin	7.81	7.81	31.3
<i>S. mutans</i> 10449	None	15.6	62.5	250
	Citrate	0.98	3.91	31.3
	Fluoride	31.3	125	125
	Oxacillin	1.95	7.81	31.3
	Streptomycin	15.6	500	250
<i>S. sanguis</i> 10556	None	15.6	7.81	15.6
	Citrate	3.91	0.98	0.98
	Fluoride	15.6	15.6	15.6
	Oxacillin	7.81	0.98	1.95
	Streptomycin	31.3	15.6	15.6
<i>S. sobrinus</i> 6715	None	15.6	7.81	62.5
	Citrate	3.91	1.95	15.6
	Fluoride	15.6	7.81	62.5
	Oxacillin	3.91	3.91	7.81
	Streptomycin	15.6	31.3	31.3

*Growth was in TSB supplemented with 5 mM sodium fluoride, 20 mM sodium citrate, oxacillin 0.05 mg/L or streptomycin 10 mg/L.

Fig. 1. Aggregation of oral streptococci by Con A showing that Con A readily aggregated *S. sobrinus* (●) 6715 and only slowly aggregated *S. cricetus* AHT (○) and *S. sanguis* 10556 (▲). **Fig. 2.** Aggregation of oral streptococci by wheat-germ agglutinin. Results in Table 2 revealed that both *S. mutans* 10449 and *S. sanguis* 10556 were aggregated by WGA. Kinetic measurements of the interaction showed that *S. sanguis* (●) was rapidly aggregated by the lectin but *S. mutans* (○) was not. **Fig. 3.** Aggregation of oral streptococci by *Helix pomatia* lectin. The HPA readily aggregated *S. sanguis* 10556 (●) and only slowly aggregated *S. sobrinus* 6715 (○) and *S. cricetus* AHT (▲). The lectin failed to agglutinate *S. mutans* 10449 (△).

Discussion

Results from this study reveal that oral streptococci can be aggregated by soluble lectins, although the aggregation patterns are unpredictable within species, e.g., variation occurred among strains of *S. mutans* (Table 2). It is known that isolates of a given species may yield their own unique aggregation patterns with lectins, and these unique patterns can be employed in epidemiological studies [8, 9].

Another interesting finding of the study is that lectins of very similar specificities may or may not aggregate a particular streptococcal strain. Wheat-germ agglutinin has a mol.wt of 43 200 and binds terminal β -N-

acetylglucosamine. On the other hand, the lectin from *Solanum tuberosum* has a mol.wt of 100 000 and also reacts with GlcNAc, but preferentially with (GlcNAc)₃. Furthermore, the lectin GSA-II from *Griffonia simplicifolia* has a mol.wt of 113 000 and is able to bind α - or β -linked GlcNAc. Interestingly, WGA and GSA-II aggregated *S. mutans* strains not recognised by STA. The STA, in contrast, was able to aggregate none of the *S. mutans* strains tested (Table 2). It is likely that WGA and GSA-II are complexing with PG that contains a glycan of β -linked GlcNAc and muramic acid. The latter sugar is known to bind with WGA and other GlcNAc-specific lectins [24, 25]. Sizemore *et al.* [26] suggested that WGA may be able to distinguish gram-positive (WGA-reactive) from gram-negative bacteria. It has been reported that not all gram-positive bacteria are WGA-reactive [27] and some gram-negative bacteria are aggregated by the lectin [5]. At present, it is not known why some oral streptococci do not react with lectins of similar specificities. Most oral streptococci produce cell wall-associated proteins and most produce PG-bound polysaccharides [28–31]. These surface structures may mask potential lectin receptors, or they may serve as receptors for other lectins. As discussed later, protease and protein denaturing conditions modify the extent of lectin-dependent aggregation of several streptococci.

It is known that the growth of oral streptococci in the presence of antibiotics or chelating agents causes the bacteria to increase or decrease GBL activities [32, 33]. Furthermore, an increase in GBL is associated with a decrease in surface hydrophobicity, whereas a decrease in GBL is mirrored by an increase in hydrophobicity. Streptomycin and citrate cause a decrease in GBL, whereas oxacillin causes an increase when these agents are added at subinhibitory concentrations to *S. cricetus* and *S. sobrinus* cultures. When oral streptococci were grown in the presence of these agents, and with fluoride, and tested for their reaction with lectins, the results were unpredictable. Streptomycin caused reactivity with Con A and HPA to decrease in some cases and increase in others, depending on the organism and the lectin. Citrate and oxacillin tended to lower the lectin concentrations needed for aggregation. It is possible that growth in media with citrate or oxacillin causes an increase in cell wall polysaccharide or a decrease in surface structures that may mask lectin receptors. Fluoride generally caused the bacteria to be less reactive with Con A or HPA.

The extraction or enzymic treatment of streptococci may be expected to alter lectin aggregation patterns [4, 34–38], as streptococcal surfaces possess both hydrophobic and hydrophilic molecules [36]. Proteases might be expected to remove proteins, leaving exposed PG and group polysaccharides, while sonication would remove adventitiously bound medium components or non-covalently bound secreted molecules. Guanidine

hydrochloride is a protein solvent, capable of removing loosely attached proteins and SDS is capable of removing most non-covalently attached surface structures. When the streptococci were subjected to guanidine, heat, SDS, sonication and Pronase, washed, suspended and treated with lectins, the results were predictable. In most cases, lectin reactivity was increased as determined by the decreased amount of lectin required to effect aggregation (Table 3). All treatments, except sonication, caused a decrease in the amount of lectin needed for aggregation. The results for sonication may be explained partially by the fact that ultrasound is capable of 'breaking' elongated structures. Therefore, sonication may have caused the loss of surface polysaccharides which are extended linear structures, unlike globular proteins. Gray *et al.* [14] observed that sialidase treatment and mild acid hydrolysis (which destroys sialic acids), enhance the interaction between WGA and group B streptococci. Trypsinisation also enhances the recognition of serotype-specific polysaccharides in *S. mutans* by antibodies [16]. Boiling, trypsin and acid treatments all increase the ability of lectins to aggregate streptococci, including the 'milleri' group [39].

A part of the present study was to gain a better understanding of bacterial surfaces by use of lectin probes. As pointed out earlier, some oral streptococci, when cultured in media with fluoride, citrate or antibiotics, have lowered glycan-binding lectin activities. In contrast, other antibiotics increase the expression of GBL on the surface of *S. cricetus* and *S. sobrinus*. The GBL is a protein situated on the PG of the streptococci and as far as is known, is not glycosylated and able to serve as a receptor of exogenously added lectins. In work in this laboratory, Mata [23] observed that when α -1,6 glucan was added to *S. sobrinus* 6715 or *S. cricetus* AHT, the cells became more 'hydrophilic', as determined by adhesion to plastics and hydrocarbons or by aggregation with ammonium sulphate. When α -1,6 glucan was added to *S. sobrinus* or *S. cricetus*, and the suspension was subjected to lectin aggregation, it was found that the glucan was unable to mask (or expose) lectin-reactive sites (Table 4). These results suggest that hydrophobic probes, but not lectins, may detect surface changes on a bacterium – probably because the latter can probe only for carbohydrate receptors.

Bacterial aggregation assays with lectins on a glass plate or in a plastic well are essentially end-point assays, which are free of kinetic considerations and are useful for screening. However, kinetic assays are required to obtain results to establish the relative reactivities of different bacterial species and strains with various lectins. In Fig. 1, it was shown that *S. sanguis* 10556 could be readily aggregated by HPA, whereas *S. cricetus* AHT, *S. mutans* 10449 and *S. sobrinus* 6715 were aggregated slowly. All of the bacteria were positive with HPA in plate assays (Table

2). Similar results were obtained when WGA was used to aggregate *S. sanguis* 10556 and *S. mutans* 10449. It is possible that the kinetic approach to detecting lectin-streptococcus complexes would be useful when distinguishing one streptococcal strain from another, but many additional assays will be required.

This is the first comprehensive examination of oral streptococci-lectin interactions and the results suggest that genus or species identification with a lectin probe is impossible. Nevertheless, it is clear that lectin receptors exist on all the streptococci and receptors can be exposed by chaotropic agents or protease, although this does not enhance specificity. Lectin receptors are not modified when surface proteins, such as GBLs, are complexed with ligands. The known structures [28–31] of some of the streptococcal surface polysaccharides are repeating units of 4–6 carbohydrates. Lectins with specificities for several carbohydrates linked in a sequence would be useful in defining surface characteristics of oral streptococci.

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