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Chemical Composition of Purified Cell Walls of Cariogenic Streptococci[1]

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The qualitative and quantitative chemical compositions of the cell walls of five strains of oral streptococci (four human and one rat) are presented. Presumptive evidence for glycerol teichoic acid in *Streptococcus mutans* group II cell walls is given.

In recent years, there have been several studies published on organisms that play a major role in dental caries, namely strains of *Streptococcus mutans* and *S. salivarius* (7-9). There are few studies, however, on the chemical compositions of purified cell walls of cariogenic streptococci (6). Such information is vital for an understanding of the antigenicity and the structure of these organisms. This report presents comparative quantitative analyses of cell walls isolated from five oral streptococci, including four cariogenic strains.

The organisms employed in this study are listed in Table 1. Mass cultures (20 liters) of each strain were grown in Todd-Hewitt Broth (Difco), supplemented with glucose (final concentration, 0.5%) and adjusted to *p*H 7.0. After 24 hr at 37 C, the cells were harvested and washed, and disrupted by glass beads by using a Braun Tissue Homogenizer (1). The cell walls were isolated and purified by methods described previously (11).

The qualitative chemical composition of each cell wall type was determined, after hydrolysis (2 N HCl, 100 C, 4 hr), by paper and thin-layer chromatography (4). Quantitative determinations of rhamnose, glucose, galactose, amino sugars, amino acids, and total phosphorus were done as previously described for cell walls of group D streptococci (2, 3).

Table 2 compares the cell wall compositions of three *S. mutans* strains, representing two serological groups (Table 1). Each of the strains lacks galactosamine in its walls, while possessing rhamnose, glucose, and galactose as major non-peptidoglycan components. Glucose, however, is a relatively minor sugar component of the group II cell wall (strains BHT and FA-I). The peptidoglycan moieties of these cell walls also appear to be different. Threonine is found in strain AHT walls in amounts almost equimolar to glutamate and lysine. This indicates that threonine, possibly associated with alanine, may serve as the cross-linking component of the muramyl peptide of this wall as in certain *S. cremoris* strains (12). Group II walls lack threonine, but possess large amounts of alanine which likely are involved in cross-linkage. The two groups are serologically distinct (10).

TABLE 1. *Streptococcal strains studied*[a]

Organism	Serological group[b]	Cariogenicity[c]	Source
S. mutans strain AHT	I	Active	Human
S. mutans strain BHT	II	Active	Human
S. mutans strain FA-I	II	Active	Rat
S. salivarius strain HHT	III	Active	Human
Unclassified strain CHT	IV	Inactive	Human

[a] All strains were isolated directly from the human oral cavity, except strain FA-I. The latter strain was obtained from R. J. Fitzgerald, Veterans Administration Hospital, Miami, Fla.

[b] Serological grouping was established by immunofluorescence (10).

[c] Cariogenicity (formation of dental lesions) was tested by orally infecting 19-day-old hamsters on a high sucrose diet with each strain (10).

Table 3 compares the cell to wall compositions of *S. salivarius* strain HHT (group III) and the unclassified strain CHT (group IV). Each of these strains contains small amounts of galactosamine and larger amounts of rhamnose and glucose. Galactose, however, is absent in the group IV cell

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TABLE 2. *Composition of the cell walls of S. mutans strains AHT, BHT, and FA-1*[a]

Major component[b]	Strain AHT		Strain BHT		Strain FA-1	
	micromoles/mg	microg/mg	micromoles/mg	microg/mg	micromoles/mg	microg/mg
Rhamnose	1.87	307.1	1.76	289.5	1.24	203.6
Glucose	0.55	99.1	0.14	26.0	0.13	23.4
Galactose	0.47	84.7	0.68	121.6	0.51	91.9
Glucosamine	0.34	75.2	0.37	81.6	0.29	65.0
Galactosamine	Nil	Nil	Nil	Nil	Nil	Nil
Muramic acid	0.34	99.7	0.35	102.3	0.31	90.9
Alanine	1.14	101.6	1.50	133.4	1.33	118.5
Lysine	0.41	59.9	0.40	57.8	0.36	53.2
Glutamate	0.42	61.8	0.35	51.3	0.39	56.8
Aspartate	0.05	6.7	0.02	2.8	0.15	19.3
Threonine	0.37	44.1	0.02	2.0	0.07	8.5
Glycine	0.03	2.3	0.02	1.2	0.11	8.3
Leucine	0.03	3.9	0.02	2.0	0.12	15.9
Serine	ND[c]	ND	0.02	2.0	0.08	8.4
Tyrosine	0.01	1.8	ND	ND	0.04	6.7
Phenylalanine	0.01	1.7	ND	ND	0.05	8.9
Phosphorus	0.18	5.6	0.57	17.8	0.47	14.7
Total recovery		955.2		891.3		794.0

[a] All values were corrected for sample hydration.

[b] Amino sugars were reported as acetylated derivatives.

[c] Not determined.

TABLE 3. *Composition of the cell walls of S. salivarius strain HHT and the unclassified strain CHT*[a]

Major component[b]	Strain HHT		Strain CHT	
	micromoles/mg	microg/mg	micromoles/mg	microg/mg
Rhamnose	0.90	147.8	1.46	239.7
Glucose	0.44	79.3	0.46	82.9
Galactose[c]	0.39	70.3	Nil	Nil
Glucosamine	0.29	64.1	0.32	70.8
Galactosamine	0.12	26.5	0.20	44.2
Muramic acid	0.36	105.6	0.32	93.8
Alanine	1.37	122.1	1.37	122.1
Lysine	0.42	61.4	0.42	61.4
Glutamate	0.38	55.9	0.37	54.4
Aspartate	0.10	13.3	0.10	13.3
Threonine	0.05	6.0	0.06	7.1
Glycine	0.05	3.8	0.07	5.3
Leucine	0.03	3.9	0.04	5.2
Serine	0.05	5.3	0.08	8.4
Tyrosine	0.02	3.6	0.03	5.4
Phenylalanine	0.02	3.3	0.02	3.3
Phosphorus	0.15	4.3	0.20	6.2
Total recovery		776.5		823.5

[a] All values were corrected for sample hydration.

[b] Amino sugars were reported as acetylated derivatives.

[c] Since galactosamine interferes with the Galactostat assay for galactose, it was necessary to measure the unknown amounts of this sugar in cell wall hydrolysates of strain HHT against a standard curve constructed by assaying mixtures of galactose of various concentrations, plus galactosamine, in the same concentration found in the hydrolysate.

wall, but is a major component of the group III wall. The peptidoglycan components of each strain are present in about the same amounts as shown for group II walls (Table 2).

Phosphorus is found in large amounts in group II cell walls (Table 2). This component, esterified with either glycerol or ribitol, is found in teichoic acids (5). To detect these sugar alcohols, cell walls (20 mg) were hydrolyzed (6 ml of 2 N HCl, 100 C, 4 hr) and evaporated to dryness; trimethylsilyl derivatives were formed by the method of Sweeley et al. (13). Samples were injected into a Packard Gas Chromatograph and compared to standard derivatives. Ribitol (as anhydroribitol) was absent in each of the five strains. Glycerol was detected only in cell walls of strains BHT and FA-1. These findings provide presumptive evidence for the presence of a glycerol teichoic acid in group II cell walls.

It is apparent that several basic chemical differences exist among cell walls of the four serological groups of oral streptococci studied. Qualitative and quantitative chemical differences are seen either in the carbohydrate, teichoic acid, or peptidoglycan moieties of isolated walls. Further studies to reveal the immunochemical bases of the serological differences are in progress.

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