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Chemical Characterization of a Cell Wall Antigen from *Streptococcus mutans* FA1

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The cell wall antigen from *Streptococcus mutans* (FA1) was extracted from cell walls and whole cells and was found to contain rhamnose, galactose, and glucosamine in a ratio of approximately 5:2:1.

There have been several procedures proposed for the classification of the various strains of *Streptococcus mutans*. These procedures are based on serological reactivity (5), growth characteristics (8), deoxyribonucleic acid composition (10), and mannitol-1-phosphate dehydrogenase (6). Serological techniques offer a rapid and sensitive method for the identification of the various strains of *S. mutans*. We have, therefore, undertaken a study of the extraction techniques and chemical composition of the antigen of *S. mutans* (FA1).

S. mutans (FA1) was obtained from R. J. Gibbons, Forsyth Dental Center, Boston, Mass., and from H. D. Slade, Northwestern University School of Dentistry, Chicago, Ill. The cells were cultured in Trypticase soy broth supplemented with 0.2% glucose (BBL). The culture was allowed to grow at 37 C for 18 to 24 h, and the cells were removed by centrifugation. The cells were washed three times in phosphate buffer (0.07 M, pH 7.0), and cell walls were prepared after homogenization with glass beads in a Braun homogenizer (4). The cell wall preparation was treated with ribonuclease, deoxyribonuclease, and then trypsin. The cell wall material was then extracted by using the following procedures: (i) treatment with 5% trichloroacetic acid at 90 C for 15 min (16), (ii) treatment with 5% trichloroacetic acid at 4 C for 7 days (1), and (iii) treatment with formamide at 180 C for 30 min (17). A fourth procedure (9) involved trichloroacetic acid (5%) extraction of whole cells at 90 C for 15 min. The solubilized preparation was then extracted with phenol (final concentration, 33%) at 90 C for 30 min. All solubilized material was dialyzed exhaustively against distilled water, lyophilized, and stored in a desiccator. The yields of the four extraction methods are presented in Table 1.

Antisera to *S. mutans* (FA1) was prepared by serial intravenous injections of heat-killed whole cells into female New Zealand white rabbits (7). The rabbits were bled by cardiac puncture, and the serum was prepared by standard procedures. The serological reactivity was determined by using standard Ouchterlony double-diffusion in 0.85% Ionagar. Solutions of the various preparations (1 mg/ml) were prepared and diffused against dilutions of antisera. The last dilution that showed a distinct positive serological precipitate was considered to be the titer of the antigen. As shown in Table 1, the serological titers of the various preparations were essentially identical (250 to 320). The antigenic material produced one precipitin band, suggesting that only one antigen was present in the preparations.

The chemical composition of the antigen preparation was determined and is presented in Table 2. Total carbohydrate content was determined by phenol-sulfuric acid assay (13), and rhamnose content was determined by the cysteine-sulfuric acid assay (12). Glucose, galactose, and glucosamine were determined after hydrolysis by glucose oxidase (15), galactose oxidase (14), and the Dische-Borenfreund assay

TABLE 1. *Yield and serological reactivity of cell wall polysaccharide from S. mutans (FA1)*

Extraction procedure	Yield	Titer
	μg/mg of dry cell	Reciprocal of greatest dilution of antigen
Trichloroacetic acid (5%, 90 C)	2.2	250
Trichloroacetic acid (5%, 4 C)	1.2	250
Formamide (180 C)	3.8	300
Trichloroacetic acid-phenol[a]	10.6	320

[a] Prepared from whole cells.

TABLE 2. *Composition of the cell wall antigen isolated from S. mutans (FA1)*

Extraction procedure	Rhamnose	Galactose	Glucose	Glucosamine	Total carbohydrates	Protein
Trichloroacetic acid (90 C)	57	13	3	13	81	13
Trichloroacetic acid (4 C)	43	14	5	17	67	22
Formamide (180 C)	47	10	3	21	79	14
Trichloroacetic acid-phenol[a]	57	12	6	23	79	17

[a] Prepared from whole cells.

(11), respectively. Protein was measured by using the Biuret method (18). Phosphate was determined (17) after a wet combustion in sulfuric acid. Even when 25 mg of antigenic material was used in the wet combustion, no phosphate was detected. Chemical analyses of the acid-hydrolyzed antigen were substantiated by thin-layer chromotography and by thin-layer electrophoresis in borate buffer.

Chromatographic separations of the hydrolyzed antigen preparations did not show any components migrating as either glycerol or glycerol phosphate. There is approximately 15% "protein" in these preparations which is predominately the cell wall amino acids, i.e., lysine, alanine, and glutamate. It is possible that some of the unaccounted weight of the antigen (4–11%) may be other cell wall components which were not specifically assayed for. Since 94 to 96% of the dry weight is accounted for in three of the extracts, it is not likely that there are teichoic acids present in these preparations.

Periodate oxidations (19) of the antigen preparations revealed that between 0.97 and 0.99 mole of periodate was consumed per mole of hexose (based on a weighted average of rhamnose:galactose equals 5:2). All of the galactose and approximately 95% of the rhamnose was destroyed. The concentration of glucosamine after oxidation was not measured. The galactose in the polymer was not susceptible to galactose oxidase oxidation, indicating that it may be substituted at C-6.

These results are very similar to those of Bleiweis, et al. (3) with respect to the ratio of rhamnose, galactose, and glucosamine found in the cell wall preparations of FA1. Subsequent work by Bleiweis, et al. (2) of alkaline-extracted cell walls from *S. mutans* BHT (serologically related to FA1) have yielded two antigenic polymers. One of the polymers contains rhamnose and phosphorus in approximately equal amounts, and the other contains a predominance of phosphorus over rhamnose and appears to be a glycerol teichoic acid. It appears, however, that this cell wall antigenic component isolated by trichloroacetic acid extraction is a rhamnose containing antigen rather than a teichoic acid antigen. This work confirms the preparation of an antigenic polysaccharide by trichloroacetic acid-phenol extractions used by Chionglo and Hayashi (9).

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ADDENDUM

While this manuscript was being considered for publication, Makasa and Slade described a cell wall antigen from *S. mutans* (Infect. Immunity 7:578–585), and van de Rijn and Bleiweis isolated a membrane-associated antigen from *S. mutans* (Infect. Immunity 7:795–804).

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