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Influence of Culture Medium on the Glucosyl Transferase- and Dextran-Binding Capacity of *Streptococcus mutans* 6715 Cells

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Growth of *Streptococcus mutans* 6715 in a medium containing trace amounts of sucrose or dextran promotes cell-associated glucosyl transferase activity and increases the dextran-binding capacity of the organisms.

The glucans produced by *Streptococcus mutans* have been implicated in the attachment of this microorganism to the tooth surface (6). Reports vary concerning the distribution of the glucosyl transferases responsible for glucan synthesis in cultures of *S. mutans*. Several investigators have observed the occurrence of cell-bound enzymes (5, 7, 8, 9) whereas others have reported that these enzymes were essentially completely extracellular in glucose broth cultures (11, 16). The present study describes the effect of the growth medium on the distribution of the glucosyl transferases in cultures of *S. mutans* strain 6715. In addition, the relationship of cell-bound enzyme to dextran- and sucrose-induced agglutination, and the capacity of 6715 cells to bind preformed, soluble dextran was examined.

Cultures of *S. mutans* strain 6715 were grown overnight in brain heart infusion broth (BBL), Trypticase soy broth (BBL), Trypticase soy broth containing 0.01% sucrose, a Trypticase-salts medium containing 0.2% glucose (TGB; [5]), TGB with 0.01% sucrose (TGB-S) and TGB with 0.001% dextran (Pharmacia), of molecular weight 2×10^6 (TGB-D). Washed cells harvested from these media, and their respective culture liquors, were assayed for glucosyl transferase activity with ^{14}C-labeled sucrose by the procedure of Robrish et al. (16). Samples were counted for radioactivity with a Packard model 3375 liquid scintillation spectrometer. Sodium fluoride (0.15%) was included in reaction mixtures to inhibit bacterial glycolysis and prevent glycogen synthesis (17). Preliminary experiments with [^{3}H]fructose-labeled sucrose indicated that strain 6715 formed only trace quantities of levan.

Virtually all detectable glucosyl transferase activity was extracellular when 6715 was grown in brain heart infusion broth or in Trypticase glucose broth (Table 1). However, when the organism was grown in Trypticase soy broth, 40% of the total glucosyl transferase activity was cell associated. This was apparently due to trace amounts of sucrose in the soy extract of Trypticase soy broth (1), because the addition of either 0.01% sucrose or 0.001% preformed soluble dextran to Trypticase glucose broth resulted in significant quantities of the enzyme becoming cell bound. Addition of 0.01% sucrose to Trypticase soy broth resulted in almost all of the enzyme activity becoming cell bound.

These data indicate that either preformed dextran, or small quantities of sucrose from which glucans are synthesized, must be present in the growth medium for the glucosyl transferases to become bound to 6715 cells. This is consistent with the reports of several investigators who have observed that dextran on the surface of *S. mutans* serves as a binding site for the glucosyl transferases (11, 13, 14, 16).

The ability of 6715 cells with and without bound glucosyl transferases to aggregate upon addition of dextran and sucrose was tested. Washed cell suspensions of cultures grown in media described above were incubated with 100 μg of soluble dextran (molecular weight 2×10^6) or with 1 mg of sucrose, and aggregation was determined as previously described (4). Similarly grown organisms were treated for 1 h with 1.0% trypsin (Nutritional Biochemicals Co.), washed, and suspended in buffer containing chloramphenicol (20 μg/ml) before testing for agglutination. Cells grown in Trypticase soy broth, TGB-S, or TGB-D which possessed cell-bound glucosyl transferase activity agglutinated strongly with both dextran and sucrose within 30 min, while washed cells, grown in TGB or brain heart infusion broth and possessing little or no glucosyl transferase, agglutinated more feebly with dextran and only slightly with sucrose (Table 2).

It has been previously suggested that cell-bound glucosyl transferases may serve as dextran-binding sites on the surface of *S. mutans*

TABLE 1. *Glucosyl transferase activity of Streptococcus mutans 6715 grown in different media*

Growth medium	Glucosyl transferase			
	Cell bound		Cell free	
	Units[a]	% of total	Units[a]	% of total
Brain heart infusion	1.4	1	145	99
Trypticase glucose broth	0	0	111	100
Trypticase glucose broth + 0.01% sucrose	23	30.1	53.2	69.9
Trypticase glucose broth + 0.001% dextran	22	14.6	128	85.4
Trypticase soy broth	55.6	39.8	84	60.2
Trypticase soy broth + 0.01% sucrose	145	96	6	4

[a] Micrograms of polysaccharide per hour per 10^8 cells, or supernatant fluid of 10^8 cells.

(4). However, the data obtained suggest that there is a second type of receptor for dextran present on the surface of 6715 cells which lack glucosyl transferase activity, but which still aggregate upon the addition of dextran. This is consistent with the observations of Olson et al. (14), who reported the inhibition of dextran-induced agglutination by serum antibody to whole *S. mutans* cells, but not by antibody to glucosyl transferase. However, these investigators could draw no conclusions concerning the nature of the dextran-binding site.

Mukasa and Slade (13) have reported that antibody directed against the group antigen of *S. mutans*, and antidextran antibody, inhibit the adsorption of the glucan-synthesizing enzymes to the cell surface. Since treatment with trypsin eliminated the ability of *S. mutans* 6715 to agglutinate with dextran (Table 2), the effect of trypsin treatment on the group antigen was examined. Rantz-Randall extracts (15) were prepared from standardized suspensions of control and trypsin-treated TGB-grown 6715 cells. When assayed by immunodiffusion with antisera against a group d strain (kindly performed by D. Bratthall), no difference in the intensity of the group precipitin band was detected. Thus, trypsin appears to completely destroy the dextran-binding component, but not the group carbohydrate antigenic determinant, suggesting that they are distinct. These conclusions are supported by the data of Gibbons and Fitzgerald (4) showing that heat-treated *S. mutans* do not agglutinate with dextran, and by the recent observations of Kelstrup and Funder-Nielsen (10) who reported that the dextran receptors are inactivated by both heat and papain. In addition, heat-killed cells have been shown to bind active glucosyl transferases (12).

An attempt was made to quantitate the dextran-binding capacity of cells with and without bound glucosyl transferase. ^{14}C-labeled dextran of molecular weight 20,000 was prepared with dialyzed, concentrated extracellular glucosyl transferases obtained from the culture liquor of predialyzed TGB-grown 6715 cells. The crude enzyme preparation was incubated with 225 μg of [^{14}C]sucrose with a total activity of 100 μCi and 25 mg of dextran of molecular weight 10,000 (Pharmacia). The dextran was precipitated with 2 volumes of 95% ethanol, washed, redissolved in water, and chromatographed sequentially on columns of Bio-Gel A-15 and P-30 which had been precalibrated with dextrans of known molecular weight. Fractions containing material of molecular weight 20,000 were collected for binding studies. The isolated dextran had a specific activity of 620 counts per min per μg. Low-molecular-weight dextran was used to determine the binding capacity of *S. mutans* cells because it does not induce aggregation (4).

In preliminary experiments, it was determined that maximum binding of dextran to bacterial cells occurred after 30- to 60-min incubation, and that the number of dextran molecules bound was linearly related to the number of molecules available through the range of at least 10^5 to 10^7 molecules per bacterial cell. In all experiments reported, a ratio of 10^6 dextran molecules per bacterial cell was used. Overnight cultures of *S. mutans*, *S. sanguis*, and *S. faecalis* grown in TGB and

TABLE 2. *Effect of trypsin treatment on the ability of Streptococcus mutans 6715 cells grown in different media to agglutinate after addition of sucrose and dextran*[a]

Growth medium	Additive	Agglutination[a]	
		Untreated cells	Trypsin-treated cells
Brain heart infusion	1.00 mg sucrose	0	ND[b]
	100 μg dextran	1+	ND
Trypticase glucose broth	1.0 mg sucrose	1+	0
	100 μg dextran	1+	0
Trypticase glucose broth + 0.01% sucrose	1.0 mg sucrose	4+	0
	100 μg dextran	4+	0
Trypticase glucose broth + 0.001% dextran	1.0 mg sucrose	4+	0
	100 μg dextran	4+	0
Trypticase soy broth	1.0 mg sucrose	4+	ND
	100 μg dextran	4+	ND

[a] Agglutination scored 0 (no agglutination) through 4+ (strong agglutination).

[b] ND, Not determined.

TGB-S were centrifuged, and the cells were washed in 0.01 M phosphate-buffered saline, pH 7.5. Duplicate 5-ml phosphate-buffered suspensions (pH 7.5) containing 10^9 washed bacteria and 10^{15} molecules of [^{14}C]dextran per ml were incubated in a shaking water bath. NaF (0.15%) was included in the reaction mixtures. After 1 h, the reaction mixtures were centrifuged, and the supernatants were discarded. The sedimented bacteria were washed twice in buffer, solubilized with 0.3 ml of Soluene 100 (Packard) and counted for ^{14}C.

Glucose-grown *S. mutans* 6715 which did not possess cell-bound glucosyl transferases bound about 3.7×10^3 molecules per cell, whereas 6715 cells grown in TGB-S and having cell-associated enzyme bound 17-fold more molecules (Table 3). Generally similar, but less dramatic increases in dextran binding occurred with *S. mutans* strains E49 and GF71. However, cells of *S. mutans* strain GS5 bound significant quantities of dextran when grown in either medium.

In contrast to the *S. mutans* strains, the affinity of dextran for cells of *S. sanguis* strains CM-1 and 34 and *S. faecalis* strain 1RaR was quite low. In most instances, only a few hundred molecules adsorbed to these organisms, and it is possible that this represents a nonspecific association of dextran with the bacterial cells. It is interesting that although *S. sanguis* produces dextran, the organisms do not exhibit dextran-induced agglutination (4), and the strains studied did not bind significant amounts of dextran. The feeble capacity of *S. sanguis* cells to interact with dextran may explain why dietary sucrose has less influence on their colonization of the tooth than *S. mutans* (2).

The increased number of dextran-binding sites on *S. mutans* 6715 cells grown in TGB-S could be due to either the presence of glucosyl transferases bound to their surface or to an increased synthesis of nonenzyme dextran receptors on the cell surface. The first possibility seems most likely because only trace quantities of sucrose in the medium promote cells with bound enzyme and increased dextran-binding capacity. The possible inductive effects of such small quantities of sucrose on the synthesis of glucan receptors would be limited to early generations of cells.

To account for the increased glucan binding capacity of TGB-S cells, multiple enzyme molecules would have to bind to each bound glucan molecule. Since the glucosyl transferases have been reported to be elaborated as aggregates (3, 5), an aggregate containing several enzyme molecules could bind to each glucan molecule on the cell surface. Alternatively, if individual enzyme molecules contained two or more dextran-binding sites, several individual enzyme molecules could bind to each cell surface glucan molecule. In either case, the number of cell-bound dextran receptors would be multiplied, and the cell would possess an increased capacity to bind dextran and to aggregate.

TABLE 3. *Dextran binding by Streptococcus strains*[a]

Strain	No. of molecules bound per cell ($\times 10^3$)[a]	
	TGB	TGB + 0.01% sucrose
S. mutans 6715	3.7 ± 0.6	65.5 ± 12.7
S. mutans E49	2.0 ± 0.04	9.6 ± 0.20
S. mutans GF71	1.6	4.5
S. mutans GS5	23.2	20.4
S. sanguis 34	0.5 ± 0.01	0.4 ± 0.01
S. sanguis CM1	1.1 ± 0.1	0.9 ± 0.22
S. faecalis 1RaR	1.1 ± 0.23	0.4 ± 0.08

[a] Values expressed are the averages of duplicate determinations performed at least twice, except in the case of GF71 and GS5. (10^6 dextran molecules available per cell).

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