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Adherence of *Streptococcus mutans* to Dextran Synthesized in the Presence of Extracellular Dextransucrase

HOWARD K. KURAMITSU

Department of Microbiology, Northwestern University Medical-Dental School, Chicago, Illinois 60611

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Live or heat-killed cells of *Streptococcus mutans* specifically adhere to dextran previously synthesized on glass surfaces by the action of extracellular dextransucrase.

Certain strains of *Streptococcus mutans* capable of synthesizing insoluble dextran appear to initiate the formation of dental plaque on smooth surfaces (8). Dextransucrase (EC 2.4.1.5.) from these strains catalyzes the formation of insoluble dextran from sucrose (4). This enzyme is primarily detected as an extracellular protein but has also been demonstrated in a cell-bound form (4). Recently, Mukasa and Slade (6) directly demonstrated that cell-bound dextransucrase can mediate the attachment of *S. mutans* to smooth surfaces. Previously, Gibbons and Fitzgerald (3) demonstrated that *S. mutans* could adhere to teeth coated with dextran synthesized by *Leuconostoc* ATCC 14935. This suggested that dextran synthesized on tooth surfaces by the action of the extracellular dextransucrase from *S. mutans* might also play a role in cellular adherence. This report demonstrates that *S. mutans* can specifically adhere to dextran formed on smooth surfaces by the action of the extracellular enzyme of the organism.

Human cariogenic *S. mutans* GS-5 and *S. salivarius* GS-15 were supplied by R. J. Gibbons, Harvard University Dental School. *S. mutans* strains HS-6, OMZ-176, FA-1 and *S. sanguis* 10556 were kindly supplied by H. D. Slade. All organisms were maintained and grown as previously described (5) except that *Bacillus stearothermophilus* was grown at 55 C. Dextran-coated glass surfaces were prepared by incubating 0.012 units of partially purified dextransucrase (0.12 units/mg) with 2% sucrose and saline-0.04% sodium azide (total volume 2.0 ml) in glass tubes (13 by 100 mm) inclined at a 30° angle. The enzyme was prepared after precipitation of the culture medium of *S. mutans* GS-5 with ammonium sulfate and passage of the enzyme through a Bio Gel A-15 column (2). After incubation for 18 h at 37 C, the tubes were decanted and gently washed three times with saline. Visual examination of the tubes revealed a thin film of dextran as noted previously (6). Glucose-grown cells (approximately 3×10^8 per tube), washed three times with saline and suspended in saline-sodium azide, were added to the dextran-coated tubes in a total volume of 2.0 ml. The tubes were again incubated for 18 h at 37 C at an inclined angle. Adhered cells were gently washed three times with saline and suspended vigorously in 3.0 ml of 0.5 N NaOH, and the turbidity was determined at 540 nm (7).

When washed cell suspensions of *S. mutans* GS-5 were incubated with glass surface-coated dextran synthesized in the presence of the extracellular enzyme, significant cellular adherence was observed (Fig. 1). Cellular adherence was shown to be dependent on the prior incubation of the extracellular dextransucrase together with sucrose (Table 1). The omission of either component resulted in turbidity measurements

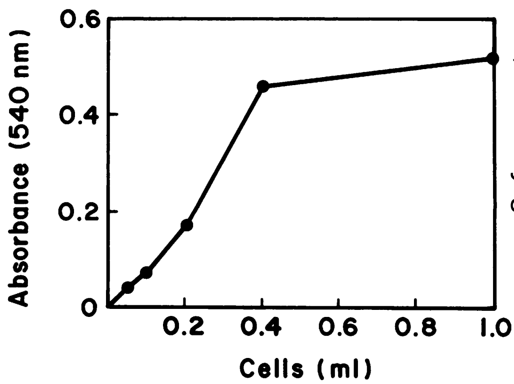

FIG. 1. *Adherence of S. mutans GS-5 to dextran-coated glass surfaces as a function of cell concentration. Adherence assays were carried out as described in the text. Absorbance values have been corrected for blank tubes lacking sucrose. The cell suspension used contained approximately 2×10^9 cells per ml.*

TABLE 1. *Requirements for cellular adherence*[a]

Dextransucrase	Sucrose	Cells	A_{540}
+	+	+	0.268
−	+	+	0.048
Boiled[b]	+	+	0.056
+	−	+	0.055
+	+	−	0.059
+	+	Boiled[b]	0.320

[a] Adherence assays were carried out as described in the text.

[b] Heated for 10 min at 100 C.

TABLE 2. *Specificity of cellular adherence*[a]

Organism	Group[b]	A_{540}[c]
S. mutans GS-5	c	0.126
S. mutans HS-6	a	0.131
S. mutans FA-1	b	0.030
S. mutans OMZ-176	d	0.055
S. salivarius GS-15		0.013
S. sanguis 10556		0
B. stearothermophilus 1503-4R		0.001

[a] Adherence assays were carried out as described in the text by using equal numbers of cells (quantitated by turbidity measurements in a Klett colorimeter).

[b] Bratthall (1) classification of *S. mutans*.

[c] Absorbance corrected for blank tubes lacking sucrose.

approximating that of NaOH solutions. Furthermore, heat-killed cells could absorb equally well to the dextran-coated tubes. This indicated that dextransucrase activity associated with the cells did not play a polymerizing role in the observed adherence.

Cellular adherence did not appear to be the result of a nonspecific trapping effect since cells of *S. salivarius*, *S. sanguis*, and *B. stearothermophilus* did not adhere significantly to the dextran-coated surfaces (Table 2). In contrast, three other strains of *S. mutans*, HS-6, FA-1, and OMZ-176, demonstrated varying degrees of adherence to the surfaces. These later differences might be the result of variations in the cell-surface recognition sites of the organisms.

These results suggest that the cell surface of *S. mutans* contains sites which specifically interact with insoluble dextran molecules. The nature of these receptor sites is of interest and currently under investigation in several laboratories. Extrapolating from these results, it seems likely that *S. mutans* can adhere specifically to tooth surfaces coated with dextran produced by the action of its extracellular dextransucrase on dietary sucrose. Furthermore, these results indicate that dextran polymer formation need not be restricted to the cell surface of *S. mutans* for cellular adherence to occur as has been suggested previously (6).

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