INFECTION AND IMMUNITY, Dec. 1977, p. 629-635
Vol. 18, No. 3

Printed in U.S.A.

Distribution of Dextransucrase in *Streptococcus mutans* and Observations on the Effect of Soluble Dextran on Dextransucrase Activities†

T. J. MONTVILLE, C. L. COONEY, AND A. J. SINSKEY*

Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received for publication 15 April 1977

Total and insoluble dextransucrase activities were measured in cell-associated and supernatant fractions of *Streptococcus mutans* GS-5 grown in several media. Although the amount of cell-associated and supernatant activity varied greatly as a function of medium, the total activity appeared constant. The distribution of dextransucrase could be altered without changing the total dextransucrase activity. This indicates that the distribution of the enzyme can be regulated independently of its synthesis. Strain GS-5 had significant cell-associated activity in media devoid of sucrose. In all cases, the ratio of insoluble to total dextransucrase activity was higher in the cell-associated fractions than in the cell-free supernatants. It is also demonstrated that exogenous soluble dextran caused a decrease in insoluble dextransucrase activity and an increase in soluble dextransucrase activity in both the cell-associated fraction and the culture supernatant. The stimulation of soluble dextran-synthesizing activity was not due to de novo synthesis. The inhibition of insoluble dextran-synthesizing activity is shown to be noncompetitive. These results support a physical rather than metabolic mechanism for the effect of soluble dextran on dextransucrase activities.

Streptococcus mutans occupies a central role in the etiology of dental caries due to its acidogenic potential and its ability to form extracellular polysaccharides. The formation of extracellular polysaccharides is of primary importance in the initiation of smooth-surface caries. The extracellular polysaccharides are synthesized by dextransucrase (EC 2.4.1.5), which incorporates the glucose moiety of sucrose into water-soluble and water-insoluble dextrans. The nature of this enzyme and the possibility that several dextransucrases may exist have been investigated by several groups (1-3, 7, 8, 11).

The dextransucrase activity that catalyzes synthesis of water-insoluble dextrans appears to be most important for the initiation of smooth-surface caries. Water-insoluble dextrans have been characterized (19) as having a greater proportion of α1-3 to α1-6 linkages than the soluble dextrans. Strains of *S. mutans* that are low in insoluble dextran production are unable to adhere to hard surfaces (4, 9) or to produce caries (18). In vitro assays of the soluble and insoluble dextransucrase activities of several *S. mutans* strains (15) demonstrated that noncariogenic and nonadherent strains had significantly lower levels of insoluble dextransucrase activity than the cariogenic strains.

The effect of soluble dextran on dextransucrase activities has been widely discussed (5, 7, 11, 14, 15). It has been suggested that the inhibition of insoluble dextransucrase activity and the concurrent stimulation of soluble dextransucrase activity is a physical rather than metabolic effect (15). In this work, a quantitative approach was taken in an attempt to further clarify the nature of this effect.

Most reports on dextransucrase investigate its activity and production in culture supernatants. Several groups (5, 12, 16) that have examined cell-associated activity have found it to be a minor component relative to the supernatant activity. Other groups (8, 14) have reported significant cell-associated activity. To reconcile these observations, soluble dextransucrase and insoluble dextransucrase activities were measured in the cell-associated fraction and supernatants of *S. mutans* cultured in several media.

Dextransucrase has both soluble and insoluble glucan-synthesizing activities, with relative proportions which may be altered in response to environmental factors (11, 15). If dextransucrase activity can also be shifted from the cell-associated to the supernatant form, the cariogenic capacity may be lessened due to the washout of

† Publication no. 3138 from the Department of Nutrition and Food Science, Massachusetts Institute of Technology.

the enzyme, and hence the cell, from the oral cavity.

MATERIALS AND METHODS

Cultures and culture conditions. *S. mutans* strain GS-5 (serotype *c*) and strain 6715 (serotype *d*) were obtained from Robert Morhart (Veterans Administration Hospital of Miami). Culture maintenance and growth were in TSYC medium, which contained Trypticase soy broth (15 g/liter), yeast extract (5 g/liter) (BBL, Bioquest Div., Becton, Dickinson & Co., Cockeysville, Md.), and cysteine (0.5 g/liter). Medium designated TH contained 30 g of Todd-Hewitt medium (Difco Laboratories, Detroit, Mich.) per liter. Medium TGB is a Trypticase-salts medium (7) containing 0.2% glucose (BBL). TGB-S (17) is TGB with 0.01% sucrose. These media were heat sterilized by autoclaving for 15 min at 15 lb/in^2 and stored at 4°C until use.

The chemically defined medium (CDM) of White et al. (20), as modified by C. H. Schein (M. S. thesis, Massachusetts Institute of Technology, Cambridge, 1976), was prepared in the manner suggested by Leslie (13). The filter-sterilized stock solutions were stored at −20°C until use. The composition of the stock solutions is given in Table 1. A filter-sterilized solution of NH_4HCO_3 and glucose in distilled water was prepared so that, upon addition of 10 ml of each of the stock solutions per liter and addition of buffer, the final concentrations of NH_4HCO_3 and glucose would be 20 mM and 5 g/liter, respectively. Phosphate buffer (1 M, pH 6.9) was added to a final concentration of 0.1 M. The complete medium was used immediately upon preparation.

Preparation of culture fractions and dextransucrase assay. *S. mutans* was grown anaerobically in a GasPak anaerobic system (BBL) for 18 to 24 h unless otherwise noted. The culture was centrifuged for 10 min at 12,000 × *g* at 4°C. The supernatant was saved for assay of supernatant dextransucrase activities. To measure cell-associated dextransucrase activity, the pellet was resuspended in an amount of phosphate buffer (0.067 M, pH 7) equal to one-third the original culture volume. Whole-culture dextransucrase activity is the sum of cell-associated and supernatant dextransucrase activities and corresponds experimentally (data not shown) to the total amount of activity in a whole culture. The ratio of cell-associated to supernatant activity (CA/Sup) was used as an indicator of the distribution of the dextransucrase activity; thus CA/Sup ratios greater than 1.0 indicate that more activity is cell associated than is in the supernatant.

TABLE 1. *Composition of vitamin and trace mineral stock solutions used in CDM*[a]

Vitamin	Trace mineral
Pyridoxine hydrochloride, 3.6	NaCl, 1.0
Nicotinic acid, 0.69	$FeSO_4 \cdot 7H_2O$, 2.78
Biotin, 0.0018	$MgSO_4 \cdot 7H_2O$, 2.5
Thiamine hydrochloride, 0.015	$MnSO_4 \cdot H_2O$, 1.69
Calcium pantothenate, 0.36	$ZnSO_4 \cdot 7H_2O$, 0.2875
p-Aminobenzoic acid, 0.003	$CuSO_4 \cdot 5H_2O$, 0.249
Cysteine hydrochloride, 10.0	$CoCl_2 \cdot 6H_2O$, 0.237
Riboflavin, 0.06	$NaMoO_4 \cdot 2H_2O$, 0.241
	$CaCl_2 \cdot 2H_2O$, 1.47

[a] 10 ml of each stock solution per liter was used. Amounts are given as milligrams per milliliter.

Insoluble dextransucrase activity is the activity that synthesizes water-insoluble glucans, and soluble dextransucrase activity is the activity that synthesizes water-soluble glucans. Both soluble and insoluble dextransucrase activities can exist in cell-associated and supernatant forms. Total dextransucrase activity is the sum of both soluble and insoluble glucan synthesis. In this study, total and insoluble dextransucrase activities were determined by a rapid filter disk assay (6, 15) that measures the incorporation of the glucose moiety of L-[*U*-^{14}C]sucrose into methanol-insoluble dextran, in the case of the total dextransucrase assay, and dextran that is insoluble in both methanol and water, in the assay for water-insoluble dextransucrase activity. (*S. mutans* strains GS-5 and 6715 have negligible fructosyltransferase activity [unpublished data].) Soluble dextransucrase activity is the difference between total and insoluble dextransucrase activities. One dextransucrase unit is defined as the amount of enzyme that converts 0.05 μmol of sucrose per min under standard conditions (10). International units can be approximated by dividing dextransucrase units by a factor of 20. Total cell counts were made by using a Petroff-Hausser counting chamber, and dextransucrase activities are expressed on a per-cell basis.

Effect of soluble dextran. *S. mutans* strain GS-5 was used to examine the effects of soluble dextran on dextransucrase activities. To investigate the effect of dextran T-10 (Pharmacia Fine Chemicals, Piscataway, N.J.) on the distribution of dextransucrase activities, a CDM culture was split into two portions. Dextran T-10 was added to a final concentration of 1% to one, and the second portion served as a control. Both were incubated for 18 h at 4°C. The cell-associated and supernatant dextransucrase activities of each were then determined.

To examine the role of de novo enzyme synthesis as an explanation for the increase in soluble dextransucrase activity, 100 μg of chloramphenicol (CAP) (17) (Sigma Chemical Co., St. Louis, Mo.) per ml was added to a portion of a TSYC culture. After 1 h, 0.5% dextran T-10 was added. A portion of the same culture with CAP but without the added dextran served as a control. After 18 h at 4°C, both portions were assayed for total and insoluble dextransucrase activity.

The dependence of the inhibition of insoluble dextransucrase activity on exogenous soluble dextran concentration was investigated by pulsing a series of TSYC-grown cultures in log phase with dextran T-10 to a final concentration of 0, 0.1, 0.5, 1, or 2%. In this case, 12 ml of culture was grown in test tubes that fit in a Klett colorimeter. The test tubes were fitted with septum caps and overlaid with nitrogen gas to achieve anaerobiosis. Three milliliters of culture was drawn just before the pulse. One milliliter of a sterile dextran solution that was 10 times the final dextran concentration was injected into the culture, and another 3-ml portion was drawn immediately after the pulse. The zero concentration was pulsed with 1 ml of sterile distilled water.

To determine the kinetics of the inhibition of insoluble dextransucrase activity by dextran T-10, cell-free

supernatants from TSYC cultures grown in the presence of 1% dextran T-10 were assayed with sucrose concentrations of 1, 2, 3, 5, 7, 10, and 15 mM in the assay mixture. Cell-free supernatants from a TSYC culture without dextran T-10 were similarly assayed as controls. The results were corrected to take into account the dilution of ^{14}C-labeled sucrose in the assay mixture by unlabeled sucrose.

Distribution of dextransucrase activities. TSYC, TH, and CDM cultures of *S. mutans* GS-5 were assayed for cell-associated and supernatant total and insoluble dextransucrase activities as previously described. To determine the effect of CDM components, the trace mineral solution and vitamin solution used in CDM were individually added to TSYC in the same concentrations as found in CDM. Cultures grown in TSYC plus the added components were assayed to determine the effect of these components on the CA/Sup ratio. To determine the level of cell-associated dextransucrase of strain GS-5 in the absence of sucrose, strain GS-5 was cultured in TGB and TGB-S and assayed as described above. For comparison, strain 6715, which has been previously characterized (17), was studied in a similar fashion.

RESULTS

Effect of soluble dextran. No significant change in the CA/Sup ratio occurred when CDM cultures of *S. mutans* GS-5 were pulsed with dextran T-10 (Table 2). However, loss of insoluble dextransucrase activity occurred in both the cell-associated and supernatant fractions. The inhibition was quantitatively identical, with a 54% decrease in the insoluble dextransucrase activity in each case.

The stimulation of soluble dextransucrase activity was not due to de novo protein synthesis. Both inhibition of insoluble dextransucrase activity and concurrent stimulation of soluble dextransucrase activity were observed in TSYC cultures to which CAP had been added (Table 3). CAP had no effect on enzyme activity (data not shown).

The concentration dependence of the dextran effect is shown in Fig. 1. This relationship was not linear on a semilog plot (not shown), indicating that the reaction does not follow first-order kinetics.

The inhibition of insoluble dextransucrase activity by soluble dextran was shown to be noncompetitive by the Lineweaver-Burk plot in Fig. 2. The apparent K_m for the insoluble dextransucrase activity in this system was 20 mM. The apparent K_i in this system was determined to be 4×10^{-2} mM.

Distribution of dextransucrase activities. Whereas the cell-associated and supernatant dextransucrase activities were growth medium dependent, the total dextransucrase activities of strain GS-5 in cultured TSYC, TH, and CDM were similar (Table 4). For example, whereas TSYC-grown cultures had about twice as much supernatant dextransucrase activity as TH-grown cultures, the TH cell-associated fractions had higher dextransucrase activities than those from TSYC cultures; the sum dextransucrase

TABLE 3. *Effect of dextran T-10 in the presence of chloramphenicol (100 μg/ml)*

Conditions	DSU[a]/liter			% Insoluble
	Total	Insoluble	Soluble	
TSYC control	600	379	221	63
TSYC + 0.5% dextran	687	261	426	38

[a] DSU, dextransucrase units.

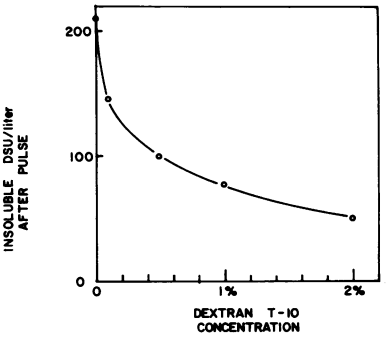

FIG. 1. *Effect of dextran T-10 concentration on whole-culture (supernatant + cell associated) insoluble dextransucrase activity. A series of log-phase TSYC cultures was pulsed with dextran T-10 to determine the effect on insoluble dextransucrase activity. DSU, Dextransucrase units.*

TABLE 2. *Effect of dextran on distribution of dextransucrase from Streptococcus mutans GS-5*

Conditions	DSU[a]/cell ($\times 10^{13}$)			CA/Sup		% Insoluble
	Total	Insoluble	Soluble	Total	Insoluble	
CDM control						
Supernatant activity	415	94	316	0.98	2.7	24
Cell-associated activity	407	269	138			66
CDM + 1% dextran						
Supernatant activity	407	46	361	0.75	2.6	11
Cell-associated activity	307	123	184			40

[a] DSU, Dextransucrase units.

activities for both media were comparable. On a per-cell basis, the dextransucrase activities in CDM were similar to those of the complex media. Growth in CDM was variable and yielded one-half to one-third as many cells as the complex media.

It has been observed by Spinell and Gibbons (17) that trace amounts of sucrose or dextran are required for dextransucrase to exist in the cell-bound form. This, however, does not explain why cells grown in CDM have a high CA/Sup ratio compared with cells grown in TSYC. To explore this observation, components of CDM were added to TSYC as noted in Materials and Methods. The effects of adding trace minerals or vitamins to TSYC are shown in Table 4. From these results, it is seen that the CA/Sup can be altered independently of changes in sum

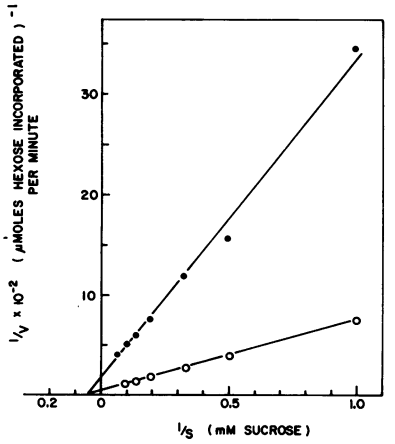

FIG. 2. *Kinetics of insoluble dextransucrase activity in cell-free TSYC supernatants with and without exogenous soluble dextran. 18-h cell-free supernatants of S. mutans cultures grown in TSYC with and without added dextran T-10 were assayed per Materials and Methods, using different sucrose concentrations in the assay mixture. Symbols: ○, Control; ●, in the presence of dextran T-10.*

dextransucrase activities. This suggests that the distribution of the enzyme can be regulated independently of its synthesis.

Additions of individual vitamins that were in the CDM (data not shown) showed no change in the CA/Sup ratio except for the addition of calcium pantothenic acid (Table 4). This effect cannot be credited to the Ca^{2+}, since the concentration of Ca^{2+} in the trace mineral solution was five times higher than the amount added as calcium pantothenic acid. Addition of this concentration of Ca^{2+} components from the trace mineral solution had no effect on the distribution of the dextransucrase activities.

Table 5 further supports the fact that sucrose is not required for dextransucrase from strain GS-5 to be cell associated. Almost half of the dextransucrase was cell associated in TGB. Addition of sucrose to TGB did increase the amount of cell-associated activity. Our results

TABLE 5. *Effect of sucrose on the distribution of dextransucrase activity*

Variable	DSU/cell (×10¹³)			
	Total	% of WC[a]	Insoluble	% of WC
6715, TGB				
Sup	337	98	136	97
CA	6	2	4	3
WC	343	100	140	100
6715, TGB-S				
Sup	83	31	26	14
CA	182	69	153	86
WC	265	100	179	100
GS-5, TGB				
Sup	387	56	259	50
CA	307	44	255	50
WC	694	100	514	100
GS-5, TGB-S				
Sup	387	49	246	40
CA	395	51	358	60
WC	778	100	604	100

[a] WC, Whole culture.

TABLE 4. *Dextransucrase activities of S. mutans GS-5 cultured in several media*

Medium	No. of trials	DSU[a]/cell (×10¹³)							
		Supernatant		Cell associated		Whole culture		CA/Sup	
		Total	Insoluble	Total	Insoluble	Total	Insoluble	Total	Insoluble
TH	2	360	160	740	490	1100	620	2.0	3.0
CDM	2	570	190	720	420	1120	600	1.2	2.2
TSYC	4	830	490	440	330	1320	820	0.5	0.7
TSYC + minerals	2	700	260	390	250	1120	510	0.5	1
TSYC + vitamins	2	530	190	530	350	1060	540	1	1.8
TSYC + calcium pantothenate	3	690	230	560	350	1250	580	0.8	1.5

[a] DSU, Dextransucrase units.

with strain 6715 agreed with those of Spinell and Gibbons (17). In marked contrast to GS-5, the dextransucrase activities from 6715 occurred almost exclusively in the supernatant when the cells were cultured in TGB. When the cells were cultured in TGB-S, the dextransucrase activities were cell associated to a greater extent than in strain GS-5. In all cases, the percentage of insoluble activity was higher in the cell-associated than in the supernatant fractions.

DISCUSSION

We have shown that the whole-culture (cell associated plus supernatant) dextransucrase activities of *S. mutans* GS-5 are similar for several media but that the dextransucrase activities are distributed between the cell surface and supernatant in different proportions. The distribution of the dextransucrase can be shifted without affecting the sum dextransucrase activities. This indicates that the distribution of dextransucrase can be regulated independently of its synthesis. We have also shown that the distribution of dextransucrase and the regulation of the distribution is strain dependent. These observations show that to study the regulation of dextransucrase, both cell-associated and supernatant activities of the particular strain must be measured.

McCabe and Smith (14) have also reported that *S. mutans* strain K1-R has only low levels of cell-associated dextransucrase activity in a medium similar to TSYC. They were able to obtain high levels of cell-associated activity by addition of sucrose to the medium. Spinell and Gibbons (17) report similar results for *S. mutans* 6715, which are confirmed in this study. We have shown, however, that *S. mutans* strain GS-5 has significant levels of cell-associated dextransucrase in TGB, CDM, and TSYC. Since TGB and CDM contain no sucrose, whereas TSYC contains traces of sucrose, and since both TSYC and CDM contain the same concentration of glucose, a factor other than sucrose must be responsible for the marked differences in the proportion of cell-associated activity of cultures grown in these media. We have shown that pantothenic acid functions in this capacity. A possible mechanism is that dextransucrase activities occur first at the cell surface, being retained there if the concentration of pantothenic acid is sufficient. If the medium is low in pantothenic acid, the enzyme is released into the supernatant and can subsequently reassociate with the cell surface in a sucrose-mediated manner.

Spinell and Gibbons (17) report that, when *S. mutans* 6715 was cultured in Trypticase soy broth, 40% of the dextransucrase was cell associated. This compares favorably with 53% of the dextransucrase activity of strain GS-5 in this study. They also observed a sucrose-mediated shift in the distribution of the enzyme. In TGB no cell-associated activity was reported for strain 6715, whereas in TGB-S 30% of the dextransucrase activity was cell associated. *S. mutans* strain GS-5 was reported to be dissimilar to the other strains studied because it bound as much dextran in TGB as in TGB-S. The ability of 6715 to bind dextran was attributed to cell-associated dextransucrase activity. Unfortunately for comparison, the proportion of cell-associated dextransucrase activity of strain GS-5 was not reported in their investigation. We have shown that strain GS-5 does have significant cell-associated activity in TGB. The cell-associated activity of strain GS-5 in TGB may indicate that pantothenic acid causes the cell association in this strain, but not in strain 6715, E49, or GF71. The cell-associated activity of strain GS-5 in TGB is consistent with their hypothesis that dextran binding is due to cell-associated dextransucrase activity.

That the ratio of insoluble to soluble dextransucrase activity is higher for the cell-associated than for the supernatant fraction is consistent with the role of insoluble dextran in the formation of caries and has been previously reported (7, 8, 15).

It is interesting to note that the variability of *S. mutans* GS-5 supernatant activities in several media is of the same magnitude as the strain-variable supernatant dextransucrase activities previously reported (15). This further complicates the extraction of generalized conclusions from specific reports in the literature.

The effect of soluble dextran on dextransucrase activities has been widely discussed (5, 7, 11, 14, 15). The results of our studies establish that the effect is rapid and occurs in the cell-associated as well as supernatant dextransucrase activities, without change in the distribution of the dextransucrase. De novo synthesis has been ruled out as the cause of the stimulation of soluble dextransucrase activity.

Guggenheim and Newbrun (8) have hypothesized that the added soluble dextran serves as a primer for soluble dextran synthesis and that, by increasing the affinity of the soluble dextransucrase for the substrate, a sort of competitive inhibition of insoluble dextransucrase activity is effected. The observation here that the inhibition of insoluble dextransucrase by exogenous dextran is noncompetitive does not support their hypothesis.

The mechanism that our data suggest is that the different dextransucrase activities are due to physical interactions of the dextran with the dextransucrase. A dextran-induced change in

molecular weight has been demonstrated by Kuramitsu (12). He reported that dextransucrase from supernatants of *S. mutans* GS-5 cultured in media containing dextran T-10 had smaller molecular weights and only soluble activity compared with the larger molecules isolated from media without dextran T-10, which synthesized both soluble and insoluble dextrans. Germaine and Schachtele (5) have also shown dextran-induced changes in the molecular weight of dextransucrase, but unfortunately did not investigate whether corresponding changes in soluble and insoluble dextransucrase activity occur.

Figure 3 schematically illustrates our current understanding of the dynamics of dextransucrase activities. Insoluble dextransucrase activity can be shifted to soluble dextransucrase activity in the presence of dextran T-10 in both the cell-associated and supernatant forms. We have shown that this is not due to changes in the distribution of the enzyme. Shifts in activity from soluble to insoluble dextran have not been demonstrated but may be possible. The dextransucrase activities are distributed between the cell surface and the supernatant. The presence of sucrose can cause a shift to the cell-associated form (14, 17). This study has shown that pantothenic acid may also play a role in the distribution of the enzyme, since its presence favors cell-associated dextransucrase activity. Kuramitsu (12) has reported that the cell-associated activity exists in two forms, one of which can be released by hypertonic salt solutions.

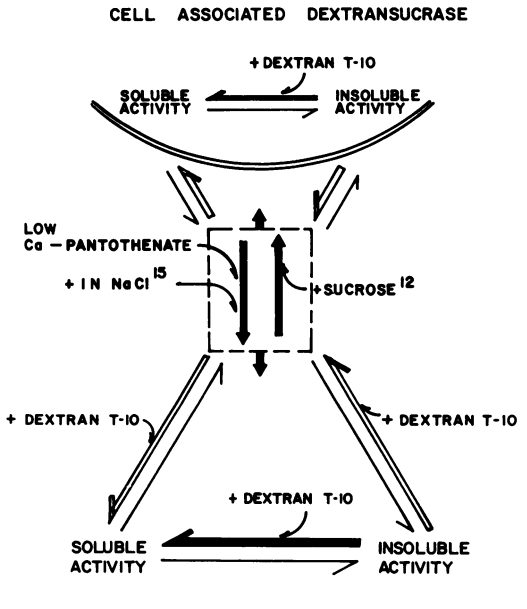

FIG. 3. *Schematic illustrating the dynamic equilibrium of dextransucrase between soluble and insoluble activity and between the cell-associated and supernatant forms. Symbols: ⇀, Conversion experimentally demonstrated; ⇌, conversion experimentally disproved; →, conversion may take place.*

In summary, dextransucrase is hypothesized to exist in a state of dynamic equilibrium between insoluble and soluble activities and cell-associated and supernatant forms. It must be emphasized that this is a composite picture that cannot be generalized to all *S. mutans* strains. *S. mutans* GS-514, for example, shows no decrease in insoluble activity in the presence of dextran T-10 (15). As we have shown, *S. mutans* GS-5 dextransucrase can be cell associated even in the absence of sucrose in the medium. Whether these differences are due to quantitative differences in affinity constants or differences in mechanisms remains to be seen. The fidelity of different *S. mutans* strains to this schematic and the determination of the extent to which various environmental factors alter these equilibria will undoubtedly be the subject of further investigations.

ACKNOWLEDGMENTS

This study was supported by Public Health Service grant DE-03976 from the National Institute of Dental Research.

LITERATURE CITED

1. **Carlsson, J., and B. Elander.** 1973. Regulation of dextransucrase formation by *Streptococcus sanguis*. Caries Res. **7**:89-101.
2. **Ciardi, J. E.** 1975. Glucosyltransferases of *Streptococcus mutans*, p. 101-110. *In* W. H. Bowen, R. J. Genco, and T. C. O'Brien (ed.), Immunologic aspects of dental caries. Information Retrieval Inc., Washington, D.C.
3. **Ciardi, J. E., G. T. Hageage, and C. C. Wittenberger.** 1976. Multicomponent nature of the glucosyltransferase system of *Streptococcus mutans*. J. Dent. Res. **55**: C87-C98.
4. **Freedman, M. L., and J. M. Tanzer.** 1974. Dissociation of plaque formation from glucan-induced agglutination in mutants of *Streptococcus mutans*. Infect. Immun. **10**:189-196.
5. **Germaine, G. R., and C. F. Schachtele.** 1976. *Streptococcus mutans* dextransucrase: mode of interaction with high-molecular-weight dextran and role in cellular aggregation. Infect. Immun. **13**:365-372.
6. **Germaine, G. R., C. F. Schachtele, and A. M. Chludzinski.** 1974. Rapid filter paper assay for dextransucrase activity from *Streptococcus mutans*. J. Dent. Res. **53**:1355-1360.
7. **Gibbons, R. J., and M. Nygaard.** 1968. Synthesis of insoluble dextran and its significance in formation of gelatinous deposits by plaque-forming Streptococci. Arch. Oral Biol. **13**:1249-1262.
8. **Guggenheim, B., and E. Newbrun.** 1969. Extracellular glucosyltransferase activity of an HS strain of *Streptococcus mutans*. Helv. Odontol. Acta **13**:84-97.
9. **Johnson, M. C., J. J. Bozzola, and I. L. Shechmeister.** 1974. Morphological study of *Streptococcus mutans* and two extracellular polysaccharide mutants. J. Bacteriol. **118**:304-311.
10. **Koepsell, H. J., and H. M. Tsuchiya.** 1952. Enzymatic synthesis of dextran. J. Bacteriol. **63**:293-295.

11. **Kuramitsu, H.** 1975. Characterization of extracellular glucosyltransferase activity of *Streptococcus mutans*. Infect. Immun. **12**:738–749.
12. **Kuramitsu, H. K.** 1975. Characterization of cell associated dextransucrase activity from glucose grown cells of *S. mutans*. Infect. Immun. **12**:738–749.
13. **Leslie, I.** 1972. Preparation of defined media, p. 1063–1070. *In* C. Long (ed.), Biochemist's handbook. E. & F. N. Spondar, London.
14. **McCabe, M. M., and E. E. Smith.** 1973. Origin of cell-associated dextransucrase of *Streptococcus mutans*. Infect. Immun. **7**:829–838.
15. **Montville, T. J., C. L. Cooney, and A. J. Sinskey.** 1977. Measurement and synthesis of insoluble and soluble dextran by *Streptococcus mutans*. J. Dent. Res. **56**:983–989.
16. **Robrish, S. A., W. Reid, and M. I. Krichevsky.** 1972. Distribution of enzymes forming polysaccharide from sucrose and the composition of extracellular polysaccharide synthesized by *Streptococcus mutans*. Appl. Microbiol. **24**:184–190.
17. **Spinell, D. M., and R. J. Gibbons.** 1974. Influence of culture medium on the glucosyl transferase and dextran-binding capacity of *Streptococcus mutans* 6715 cells. Infect. Immun. **10**:1448–1451.
18. **Tanzer, J. M., B. M. Chassy, and M. I. Krichevsky.** 1972. Sucrose metabolism by *Streptococcus mutans* SL-1. Biochim. Biophys. Acta **261**:379–387.
19. **Usui, T., H. Sugiyama, S. Seto, S. Araya, T. Nisizawa, S. Imai, and K. Kosaka.** 1975. Structural determination of glucans from *Streptococcus mutans* JC-2 (dental caries bacterium) by carbon-13 nuclear magnetic resonance. J. Biochem. **78**:225–227.
20. **White, G. E., C. L. Cooney, A. J. Sinskey, and S. A. Miller.** 1974. A defined continuous culture medium for *Streptococcus mutans*. J. Dent. Res. **53**:762.