INFECTION AND IMMUNITY, Feb. 1983, p. 762–766
0019-9567/83/020762-05$02.00/0

Vol. 39, No. 2

Immunochemical Properties of Glucosyltransferases from *Streptococcus mutans*

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Received 7 September 1982/Accepted 18 November 1982

Antiserum against purified mutansynthetase (EC 2.4.1.?) of *Streptococcus mutans* 6715 (serotype *g*), which is responsible for the synthesis of water-insoluble glucan (ISG) in the presence of both sucrose and water-soluble glucan, was prepared. The specificity of the antiserum was tested by using crude enzyme preparations (CEPs) of *S. mutans* strains of various serotypes. On immunodiffusion, the antiserum cross-reacted with CEPs from strains of serotypes *a* (HS-6 and AHT), *d* (OMZ176), and *g* (OMZ65 and KIR), but not with those from strains of serotypes *b* (BHT and FA-1) and *c* (GS-5 and Ingbritt). The antiserum inhibited the synthesis of ISG by crude or purified mutansynthetase of *S. mutans* 6715. The activities of ISG synthesis by CEPs from the strains antigenically related in the foregoing immunodiffusion were inhibited by the antiserum against strain 6715 mutansynthetase. The antiserum, however, also inhibited the enzyme activity of the strains of serotype *b*. The finding that the antiserum against purified dextransucrase of *S. mutans* HS-6 inhibited ISG synthesis by a CEP of strain HS-6 and also by CEPs of antigenically related strains suggested that dextransucrase activity is involved in ISG synthesis.

Water-insoluble glucan (ISG) synthesized by *Streptococcus mutans* from sucrose plays an important role in streptococcal colonization and dental plaque formation on smooth tooth surfaces (6). At least two kinds of extracellular glucosyltransferases (GTases) of *S. mutans* have been described on the basis of the formation of glucosyl linkages catalyzed by them. One, which synthesizes water-soluble glucan (SG) mainly consisting of α-1,6 glucosyl linkages, has been characterized as dextransucrase (EC 2.4.1.5.) (2). Using dextransucrase purified from *S. mutans* HS-6 (serotype *a*) and its homologous antiserum (anti-DS), we have examined the specificity of the antiserum (3).

Recently we have purified the other GTase from *S. mutans* 6715, named it mutansynthetase (EC 2.4.1.?), and demonstrated that it synthesizes ISG consisting mainly of α-1,3 linkages in the presence of both sucrose and SG (4). The enzyme exhibits little activity in the absence of SG.

Although workers in several laboratories have examined the antigenic properties of GTases from *S. mutans* (3, 7–11, 13), there are few data available concerning the antigenic specificity of mutansynthetase; furthermore, these previous investigations utilizing antisera against ISG-synthesizing GTase (7–10, 13) apparently were not carried out with homogeneous preparations. These results prompted us to prepare the antiserum against mutansynthetase of *S. mutans* 6715 (anti-MS) and to examine its specificity. We tested the inhibitory action of the antiserum on ISG synthesis by crude enzyme preparations (CEPs) of *S. mutans* strains of various serotypes and also examined whether anti-DS inhibited ISG synthesis.

MATERIALS AND METHODS

Preparation of crude enzyme and purified mutansynthetase. CEPs of various *S. mutans* strains were obtained by ammonium sulfate precipitation from culture fluids as previously described (2). The strains used were of serotypes *a* (HS-6 and AHT), *b* (BHT and FA-1), *c* (GS-5 and Ingbritt), *d* (OMZ176), and *g* (OMZ65, KIR, and 6715).

Mutansynthetase was purified from concentrated culture fluids of *S. mutans* 6715 grown in chemically defined fructose medium as previously described (4). The purified mutansynthetase, which was shown to be electrophoretically homogeneous, was used as an immunogen.

Preparation of antisera against purified mutansynthetase and dextransucrase. Anti-MS was prepared in two rabbits by intramuscular injection of purified mutansynthetase (69 μg) mixed with an equal volume of Freund complete adjuvant (Difco Laboratories, De-

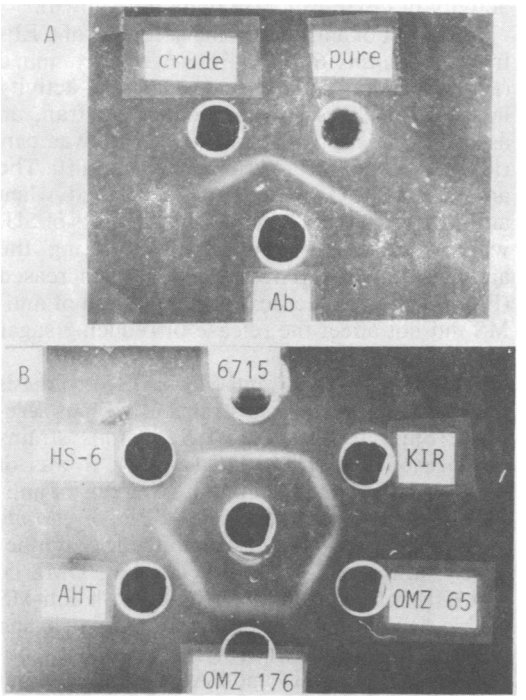

FIG. 1. Examination of specificity of anti-MS by immunodiffusion. (A) Samples (10 μl) of purified mutansynthetase (pure), *S. mutans* 6715 CEP (crude), and anti-MS (Ab) were added to the wells. (B) The center well was filled with 10 μl of anti-MS, and each outer well was filled with 10 μl of the respective crude GTase preparation of *S. mutans*. Crude GTase preparations of *S. mutans* serotype *b* (BHT and FA-1) and *c* (GS-5 and Ingbritt) strains did not react with anti-MS. Purified mutansynthetase (3 μg) and protein of CEPs (about 100 μg) were applied.

troit, Mich.). Each animal received three injections of antigen at intervals of 3 weeks and was bled completely 10 days after the final injection. The anti-MS globulin fraction was prepared from pooled antisera by repeated precipitations with 33% saturated ammonium sulfate as previously described (2). The globulin fraction was concentrated to about one-half of the pooled serum.

The anti-DS used in the present studies has been stored at −20°C for about 10 years. The preparation of anti-DS was described previously (2).

Enzyme assay. GTase activities were measured by determining total glucan and SG synthesized or reducing sugar released from sucrose or both, as previously described (4). The purified mutansynthetase activity was determined in the presence of dextran T10 (final concentration of 1.0 mg/ml; Pharmacia Fine Chemicals, Uppsala, Sweden). The amount of dextran T10 added as substrate subtracted from the total glucan equals the amount of glucan product formed de novo. We designated these portions de novo-synthesized glucan.

The activities of CEPs were determined without the addition of dextran T10. When the effects of antiserum on GTase activity were determined, enzyme preparations were preincubated with and without antiserum for 30 min at 37°C, before the addition of other components for enzyme activity. In this connection, preimmune sera had accelerated glucan synthesis of GTase preparations.

Immunodiffusion. Immunodiffusion tests were performed in 1% Noble agar (Difco) as previously described (3).

RESULTS

Antigenic analysis of mutansynthetase by agar gel immunodiffusion. Anti-MS produced a single precipitin band with crude and purified mutansynthetase preparations of *S. mutans* 6715 (Fig. 1A), whereas it did not react with dextransucrase preparations from *S. mutans* 6715 (data not shown), indicating that mutansynthetase was antigenically different from dextransucrase.

The ability of anti-MS to cross-react with CEPs from various strains of *S. mutans* was tested by immunodiffusion (Fig. 1B). The precipitin line with a CEP of strain 6715 fused with those of CEPs from strains AHT, OMZ176, OMZ65, and KIR, whereas it formed a spur with the line of strain HS-6. CEPs of strains BHT, FA-1, GS-5, and Ingbritt, however, gave no visible precipitin line with anti-MS.

Effect of anti-MS on GTase activities. Figure 2 shows the effects of increasing concentrations of anti-MS on purified mutansynthetase from *S. mutans* 6715 in the presence of dextran T10. The synthesis of ISG was completely inhibited by the specific antibody, whereas the release of reducing sugar was not completely inhibited. Since

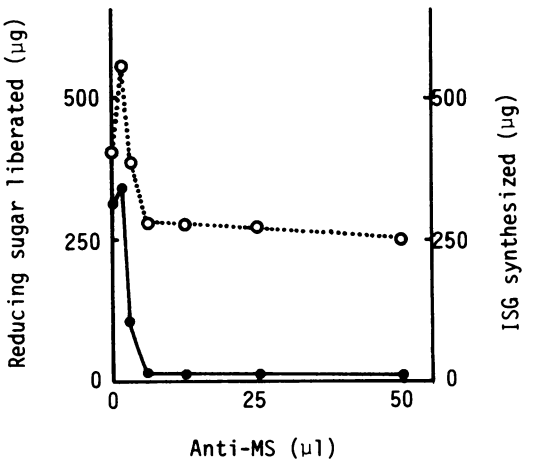

FIG. 2. Effects of anti-MS on activity of mutansynthetase of *S. mutans* 6715. Purified mutansynthetase (3.7 μg) was incubated with various amounts of anti-MS in the presence of dextran T10 (1.0 mg/ml) and sucrose (0.125 M). After 60 min of incubation, the amounts of reducing sugar (○) and ISG (●) were determined.

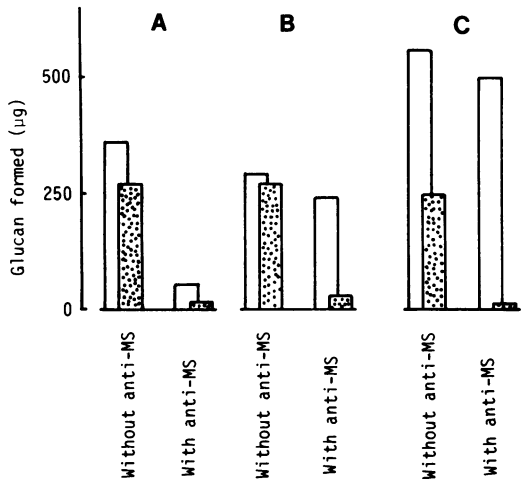

FIG. 3. Inhibition of glucan synthesis by anti-MS. Purified mutansynthetase of *S. mutans* 6715 (A) was incubated with and without anti-MS (50 μl) in the presence of dextran T10 and sucrose. After 60 min of incubation, the amounts of de novo glucan (▭) and ISG (▨) were determined. CEPs from *S. mutans* 6715 (B) and HS-6 (C) were incubated with and without anti-MS (50 μl) in the presence of sucrose. After 60 min of incubation, the amounts of total glucan (▭) and ISG (▨) were determined.

these results could not rule out the synthesis of SG by purified mutansynthetase in the presence of anti-MS, an examination was made to determine whether the de novo glucan synthesis would be inhibited by the addition of anti-MS. The de novo glucan synthesis was inhibited (Fig. 3A). On the other hand, a preliminary experiment showed that anti-MS did not inhibit the activity of dextransucrase (data not shown).

The effect of anti-MS on the activities of CEPs from *S. mutans* 6715 and HS-6 was examined (Fig. 3B and C). Since dextransucrase activity involved in CEPs should produce dextran, an assay of glucan synthesized by CEPs was carried out without the addition of dextran T10. The amounts of ISG synthesized decreased when anti-MS was added, whereas the amounts of SG, which were determined by subtracting the amounts of ISG from total glucan, increased (Fig. 3B and C). Moreover, the addition of anti-MS did not affect the release of reducing sugar (data not shown).

Inhibitory effects of anti-MS on ISG synthesis by CEPs from *S. mutans* strains of various serotypes. Since CEPs of most *S. mutans* strains cross-reacted with anti-MS (Fig. 1), it was of interest to examine the inhibitory effects of anti-MS on the synthesis of ISG by CEPs of various strains. Anti-MS markedly inhibited ISG synthesis by strains HS-6, AHT, OMZ176, OMZ65, and KIR, which had cross-reacted with anti-MS (Table 1). Among the preparations which had not reacted with anti-MS, inhibition was observed in strains BHT and FA-1 (serotype *b*) but not in serotype *c* strains.

Inhibitory effect of anti-DS on ISG synthesis by CEPs from *S. mutans* strains of various serotypes. Anti-DS inhibits the synthesis of SG by purified dextransucrase of strain HS-6 (3). Since purified mutansynthetase synthesizes ISG in the presence of SG, it is of interest to examine whether the synthesis of ISG is inhibited by the addition of anti-DS to a CEP.

Anti-DS did not inhibit, but rather accelerated, the activity of purified mutansynthetase of

TABLE 1. Inhibition by anti-MS of ISG synthesis by CEPs from *S. mutans* strains of various serotypes[a]

Serotype	Strain	ISG synthesized (μg):		
		Without anti-MS	With anti-MS	% Inhibition of ISG synthesized
a	HS-6	98.7	1.9	97
	AHT	232.5	18.3	92
b	BHT	101.8	15.6	85
	FA-1	153.6	48.8	68
c	GS-5	146.8	262.8	−79
	Ingbritt	155.5	278.2	−79
d	OMZ176	210.6	26.2	88
g	OMZ65	165.9	3.2	98
	KIR	127.8	3.2	97
	6715	180.8	17.3	90

[a] After 60 min of incubation, the amount of ISG formed was determined by the phenol-sulfuric acid method with glucose as a standard. A 50 μl sample of anti-MS was used.

TABLE 2. Inhibition by anti-DS of ISG synthesis by CEPs from *S. mutans* strains of various serotypes[a]

Serotype	Strain	% Inhibition of ISG synthesized
a	HS-6	84
b	BHT	70
c	GS-5	−86
d	OMZ176	77
g	OMZ65	86
g	KIR	88
g	6715	56

[a] The enzyme assay system was the same as described for Table 1 except that anti-DS serum was used.

strain 6715 (data not shown). The inhibitory effects of anti-DS on ISG synthesis by CEPs from the representative strains of the different serotypes are illustrated in Table 2. Synthesis of ISG by CEPs from serotypes *a*, *b*, *d*, and *g* was inhibited by the addition of anti-DS. Inhibition was not observed in the CEP from strain GS-5 (serotype *c*).

DISCUSSION

Although the antigenic properties of GTases from *S. mutans* strains have been studied by workers in several laboratories (3, 7–12), few data are available concerning the antigenic specificities of purified mutansynthetase. Since antiserum against purified mutansynthetase from *S. mutans* 6715 was obtained, comparative studies of immunological properties of mutansynthetase among various strains of *S. mutans* were carried out.

The antigenic specificity of mutansynthetase of strain 6715 (serotype *g*) was shared on immunodiffusion with those of strains of serotypes *a* (HS-6 and AHT), *d* (OMZ176), and *g* (OMZ65 and KIR) but not with those of strains of serotypes *b* (BHT and FA-1) and *c* (GS-5 and Ingbritt). These results suggest that the antigenic relationships of mutansynthetase among strains of various serotypes are similar to those of dextransucrase, which have been described previously (3).

S. mutans AHT has previously been assigned to serotype *a* (1). However, as shown in Fig. 1, the precipitin line formed between the CEP of strain AHT and anti-MS fused with those formed by CEPs of serotype *d* and *g* strains but showed a spur formation with the line formed by the CEP of strain HS-6 (serotype *a*), indicating that strain AHT belonged to serotype *d* or *g*. This supports the recent finding that strain AHT is of serotype *g* (5).

Table 1 shows the inhibitory effects of anti-MS on ISG synthesis in strains of various serotypes. ISG-synthesizing activity of CEPs from strains antigenically related on immunodiffusion (Fig. 1B) was inhibited by anti-MS. The antiserum also inhibited the activity of the strains of serotype *b*, CEPs of which did not cross-react with anti-MS. The reason why anti-MS did not form precipitin bands with CEPs of serotype *b* is not clear. Anti-DS also does not cross-react with CEPs of serotype *b* on immunodiffusion (3).

We have shown previously that mutansynthetase transfers the glucose moiety of sucrose to C-3 positions of glucosyl residues of SG and then elongates α-1,3 glucosyl linkages in the branched side chains; thus, the processed SG is converted to ISG (4). The present study showed that anti-MS inhibited de novo glucan synthesis by purified mutansynthetase of *S. mutans* 6715 in the presence of dextran T10 (Fig. 3A), but did not inhibit the release of reducing sugar (Fig. 2). These findings indicate that mutansynthetase transferred the glucose moiety of sucrose to water but not to SG in the presence of anti-MS.

CEPs of *S. mutans* synthesize ISG without the addition of SG. It might be that dextransucrase in CEPs primarily synthesizes SG, to which mutansynthetase transfers the glucose moiety of sucrose. We have domonstrated previously (3) that anti-DS inhibits SG synthesis by dextransucrase of strain HS-6 and antigenically related strains of other serotypes. Therefore, it might be expected that the addition of anti-DS to CEPs of those strains would result in the inhibition of ISG synthesis. The results of Table 2 support the foregoing presumption. Here, too, anti-DS inhibited ISG-synthesizing activity in the CEP of strain BHT (serotype *b*), which does not cross-react with anti-DS on immunodiffusion (3).

The ISG synthesis by CEPs of serotype *c* strains was increased by the addition of anti-MS and anti-DS fractions (Tables 1 and 2). In addition, we observed that preimmune sera caused an elevation of the activities of CEPs of *S. mutans*, purified mutansynthetase of strain 6715, and dextransucrase of strain HS-6 (data not shown). Therefore, the stimulation of ISG synthesis on serotype *c* strains would not be caused by the specific anti-MS and anti-DS antibodies but by other components in the sera. Schachtele et al. have demonstrated that phospholipids in human sera enhance glucan synthesis by GTase from *S. mutans* (12).

In CEPs of *S. mutans*, either anti-DS or anti-MS inhibits ISG synthesis, supporting the hypothesis of ISG synthesis in *S. mutans* that SG synthesized from sucrose by dextransucrase behaves as an acceptor of the glucose moiety of sucrose transferred by mutansynthetase to synthesize ISG.

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