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SPECIFIC INHIBITION OF GLUCOSYLTRANSFERASE OF *Streptococcus mutans*

MASAKAZU INOUE* AND ERIC E. SMITH
Department of Biochemistry, University of Miami School of Medicine, Miami, Florida 33152 (U.S.A.)
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ABSTRACT

Clinical dextran, partially oxidized with sodium periodate, acts as a potent inhibitor of the extracellular glucosyltransferases of several cariogenic strains of oral *Streptococcus mutans*. Preincubation with oxidized dextran resulted in a rapid loss of up to 80% of the ability of the enzyme preparation to synthesize polysaccharide from sucrose, but there was no loss of enzyme activity when the oxidized dextrans were reduced with sodium borohydride before preincubation with enzyme. The presence of unoxidized clinical dextran during the preincubation period afforded the enzymes protection against inhibition by partially-oxidized dextran, but clinical dextran did not readily restore activity when it was added after incubation of the enzyme with oxidized polysaccharide. Fructosyltransferase, and glycogen and starch phosphorylase, activities were not inhibited by oxidized dextran, and the bacterial glucosyltransferases were not inhibited by partially oxidized glycogen and amylose. It is proposed that the potent and specific inhibition of glucosyltransferase by oxidized dextran results from the interaction of dialdehyde groups with reactive functional groups close to the dextran-binding site of the enzyme.

INTRODUCTION

The role of *Streptococcus mutans* as an etiological agent in dental caries is well documented, and the secretion of extracellular glucosyltransferases (EC 2.4.1.5) that synthesize water-insoluble α-D-glucans from dietary sucrose has been related to the ability of a number of strains of *S. mutans* to adhere to tooth surfaces and form tenacious plaques[1–5]. Acid production within the bacterial plaques then leads to demineralization and eventual destruction of enamel, so that the tooth becomes susceptible to infection and is eventually destroyed by other invasive microorganisms.

Modification of the structure of the α-D-glucans synthesized by cariogenic organisms has had varied success in decreasing plaque-deposition and the incidence of dental caries in experimental animals fed diets high in sucrose[6–12]. Thus, rats

*Present address: Department of Preventive Medicine, Kyushu University School of Dentistry, Fukuoka, Japan.

treated with mutanase [(1→3)-α-D-glucanohydrolase] exhibited lower caries scores than untreated animals[13], presumably because enzymic hydrolysis of a proportion of the water-insoluble polysaccharides leads to dissolution of plaque. The addition of water-soluble, low-molecular-weight dextran to sucrose diets also caused some decrease in experimentally induced caries in hamsters[14]. Soluble dextran, by acting as an acceptor in the glucosyltransferase reaction[15], decreases the initial synthesis of water-insoluble α-D-glucan[16], and so may favor the formation of less-adhesive plaques.

The reason for only limited success in these attempts to prevent the development of experimentally induced caries may be that the agents employed serve to modify the structure of the α-D-glucan either during (soluble dextran in diet), or after (enzymic hydrolysis) its synthesis from sucrose. Because specific agents capable of inhibiting the initial synthesis of α-D-glucans from sucrose might be expected to be more successful in preventing plaque deposition, we describe in this paper the preparation and *in vitro* action of an oxidized dextran that specifically inhibits the synthesis of α-D-glucan by the crude glucosyltransferase isolated from a number of cariogenic strains of *S. mutans*. Some of these results were reported in preliminary communications[17,18].

MATERIALS AND METHODS

Materials. — Clinical dextran (mol. wt. 60,000–90,000) and amylose (degree of polymerization, 300) were products of Nutritional Biochemical Corporation, Cleveland, Ohio, and glycogen was a laboratory sample isolated from rabbit liver. Sucrose (Fisher Scientific Co.) was dialyzed before use through a cellophane membrane to remove traces of dextran. [^{14}C]Sucrose([U-^{14}C]glucosyl) and [^{3}H]sucrose([1-^{3}H]-fructosyl) were products of New England Nuclear Corporation. Rabbit-liver phosphorylase *a* was a gift from Dr. D. Killilea, Department of Biochemistry, University of Miami. Starch phosphorylase was partially purified from a potato extract by absorption onto, and elution from, a butylamine–Sepharose column. The bacterial strains used were kindly made available by Drs. R. Fitzgerald and R. Cowman, Dental Research Unit, Veterans Administration Hospital, Miami, Florida. Glucosyltransferase and fructosyltransferase preparations were obtained as crude ammonium sulfate fractions as described later. A highly purified fraction of glucosyltransferase from *S. mutans* 6715-49 was prepared as described by McCabe and Smith[19].

Methods. — Concentrations of solutions of polysaccharides and sucrose were determined by the phenol–sulfuric acid method[20], and the release of reducing sugars was measured by a modified Nelson procedure[21]. The activity of glycogen phosphorylase *a* was assayed as described by Hedrick and Fischer[22] and potato phosphorylase activity by the method of Whelan and Bailey[23]. Protein concentration was determined by the Lowry method[24].

Preparation of glucosyltransferase and fructosyltransferase. — Unless stated otherwise, strains of *Streptococcus* were grown at 37° under 19:1 nitrogen–carbon

dioxide in broth containing Trypticase BBL (20 g/L), yeast extract (49 g/L), D-glucose (10 g/L), and sodium carbonate (4 g/L). The pH was adjusted to 7.0 with concentrated hydrochloric acid before sterilization. After sterilization, cysteine (100 mg/L) was added and the broth was inoculated with 10% (by volume) of a stationary-phase culture of the cells. After 18 h, the culture was centrifuged at 13,000*g* for 15 min and the cell-free liquor was adjusted to 50% saturation with crystalline ammonium sulfate and kept for 4 h. The precipitate was collected by centrifugation (13,000*g*, 20 min), dissolved in 0.02% sodium azide (2.5% of the original culture-volume), and dialyzed against the same solution overnight. All procedures were conducted at 4°. Enzyme prepared in this way is referred to as the crude ammonium sulfate fraction. Glucosyltransferase activity was assayed by measurement of D-[^{14}C]glucose incorporated from [^{14}C]sucrose([U-^{14}C]glucosyl) into 70% ethanol–insoluble polysaccharide, as described previously[25]. Fructosyltransferase was assayed by measuring [^{3}H]fructose incorporated from [^{3}H]sucrose([1-^{3}H]fructosyl) into 70% ethanol-insoluble polysaccharide under similar conditions. One unit of glucosyltransferase or fructosyltransferase is defined as the amount of enzyme that incorporates 1 μmol of hexose into ethanol-insoluble polysaccharide in 1 min under the conditions defined. A typical glucosyltransferase preparation contained 0.8 International Units per 25 mg of protein. A portion of the ammonium sulfate enzyme-fraction obtained from a culture of *S. mutans* 6715-49 was used for further purification by affinity and hydrophobic chromatography[19].

Periodate-oxidation of clinical dextran and other α-D-glucans. — Solutions of clinical dextran (mol. wt. 60,000–90,000, 10 mg/mL in distilled water) were cooled to 4° and an equal volume of cold solutions containing different concentrations of sodium metaperiodate (0.62, 3.1, 6.2, and 62 μM) was added to each with rapid stirring. The solutions were kept in the dark for 14 h at 25°, and the oxidized dextrans were dialyzed exhaustively against tap water and distilled water, and then freeze dried. A solution of unoxidized clinical dextran (5 mg/mL) was also dialyzed and freeze-dried. Solutions of amylose and glycogen (10 mg/mL) were similarly oxidized by the addition of equal volumes of 6.2 μM sodium metaperiodate. The freeze-dried powders were dissolved in 0.02% sodium azide at appropriate concentrations. Portions of the oxidized dextran preparations were reduced with sodium borohydride for 14 h at 25° as described by Goldstein *et al.*[26].

The inhibitions of glucosyltransferase by dextrans partially oxidized under the foregoing conditions are reproducible, but the exact degree of oxidation of dextran by periodate was not determined in these studies. The values for percent of oxidation given in the results are based on the assumption that all of the periodate in oxidation mixtures is consumed and that no D-glucose residues of dextran are oxidized by more than one periodate ion. Oxidation of glucose residues in a dextran by an equimolar proportion of periodate is arbitrarily assumed to effect oxidation of all glucose residues (100% oxidation). This assumption, is, of course, not entirely valid, and current, detailed structural investigations on such partially oxidized clinical dextrans will be reported later.

Assay of the extent of inhibition of glucosyltransferase. — A number of freshly prepared solutions of 10%-oxidized dextran failed to inhibit glucosyltransferase activity, and the maximum extent of enzyme inhibition by other preparations varied greatly. When the solutions of oxidized dextran were heated for 15 min to 90° and cooled, their inhibitory activity was fully expressed, and they remained active when stored for 14 days at 4°. Therefore, unless otherwise stated, solutions of inhibitors were routinely heated to 90° and then cooled. Under standard conditions, glucosyltransferase (~0.01 unit) and oxidized glucans (12.5 μg) were preincubated for 15 min at 37° in a solution (75 μL) containing 0.02% sodium azide and 50mM sodium phosphate, pH 6.0. The solutions were then placed in ice in readiness for measurement of their glucosyltransferase activity. Precooled substrate solution (50 μL), containing [^{14}C]sucrose (1 mg, 2.5 × 10^{-8} Ci), clinical dextran (25 μg), 0.02% sodium azide, and 50mM sodium phosphate (pH 6.0), was added to the cold, preincubation mixtures (75 μL), which were then incubated at 37°. After a suitable time, samples (50 μL) were removed and spotted onto filter-paper squares that were immediately submerged in 70% aqueous ethanol. After two washings (10 min) in the ethanol solution, papers were washed in acetone and then dried, and the amount of [^{14}C]glucose incorporated into the polysaccharide that remained on the papers was measured in a scintillation spectrometer.

RESULTS

The glucosyltransferase activity of *S. mutans* 6715-49 was significantly inhibited when the enzyme was preincubated with clinical dextran that had been partially oxidized with periodate (Table I). Dextran treated with 0.1 mol of periodate per mol of glucose residues (10% oxidized) was the most potent inhibitor of enzyme activity, whereas dextran treated with 1 mol of periodate per mol of glucose residues (100% oxidized) was much less inhibitory. Enzyme assays based on the release of fructose (a copper-reducing sugar) from the sucrose substrate revealed the same extents of inhibition of the crude enzyme-preparations by the oxidized dextrans (data not shown), and also showed that a glucosyltransferase preparation, purified about 400-fold by fractionation on columns of insoluble dextran and butylamine–Sepharose, was also inhibited (Table I).

Glucosyltransferase activity decreased rapidly during the first 10 min of preincubation at pH 6.0 with 10%-oxidized dextran, and only 17% of the original activity remained after preincubation for 60 min (Fig. 1). We were unable to assess the real effect of high concentrations of 10%-oxidized dextran on enzyme activity, because unoxidized dextran also appeared to inhibit the enzyme activity under these conditions (Table II). The apparent inhibition by unoxidized dextran is the result of the transfer of a large amount of dextran to the enzyme-assay mixture[15], but it is clear that the enzyme is significantly inhibited by much lower concentrations of 10%-oxidized dextran.

TABLE I

INHIBITION OF *Streptococcus mutans* GLUCOSYLTRANSFERASE AFTER PREINCUBATION WITH PERIODATE-OXIDIZED CLINICAL DEXTRAN[a]

Extent of oxidation of clinical dextran[b] *(%)*	*Relative enzyme activity after preincubation (c.p.m. incorporated)*	*Inhibition*[c] *(%)*
0	1410	0 (0)
1	1350	4 (13)
5	940	33 (44)
10	430	70 (42)
100	1300	8 (5)
No dextran	1350	4 (9)

[a]Enzyme was preincubated for 10 min at pH 6.0 and 37° with or without oxidized dextran (170 μg/mL), and the enzyme activity was determined under standard conditions as described in Materials and Methods. [b]Percent oxidation of the dextran in the preincubation mixture is based on the proportions of sodium metaperiodate and dextran used in the oxidation procedure. An equimolar proportion of periodate and dextran glucose residues is arbitrarily taken as giving 100% oxidation. [c]Numbers in parentheses are the corresponding inhibitions of the activity of a 400-fold purified enzyme, as measured by the release of reducing sugars.

TABLE II

INHIBITION OF *S. mutans* GLUCOSYLTRANSFERASE PREINCUBATED WITH DIFFERENT CONCENTRATIONS OF OXIDIZED AND UNOXIDIZED DEXTRAN

Concentration of dextran in preincubation mixture (mg/mL)	*Enzyme activity after preincubation with oxidized or unoxidized dextran*[a]					
	10% Oxidized		*100% Oxidized*		*Unoxidized*	
	[^{14}C]*Glucose incorporated (c.p.m.)*	*Inhibition*[b] *(%)*	[^{14}C]*Glucose incorporated (c.p.m.)*	*Inhibition*[b] *(%)*	[^{14}C]*Glucose incorporated (c.p.m.)*	*Inhibition*[b] *(%)*
0.25	540	58	1270	2	1300	0
0.50	420	68	1200	8	1260	3
1.0	410	68	1150	12	940	28
2.0	370	72	1050	19	670	48
5.0	340	74	970	25	530	59

[a]Enzyme was preincubated for 10 min at pH 6.0 and 37° with the indicated concentration of oxidized and unoxidized dextrans, and activity was determined under standard conditions as described in Materials and Methods. [b]Inhibition is expressed as the percent decrease in activity compared to that of enzyme preincubated with unoxidized dextran at a concentration of 0.25 mg/mL.

Different preparations of 10%-oxidized dextrans, dissolved in 0.02% sodium azide (5 mg/mL), did not exhibit the same ability to inhibit glucosyltransferase. The effect of storage and heat on a diluted solution (1 mg/mL) of one of the 10%-oxidized dextran preparations is shown in Fig. 2. Storage of the dilute solution for

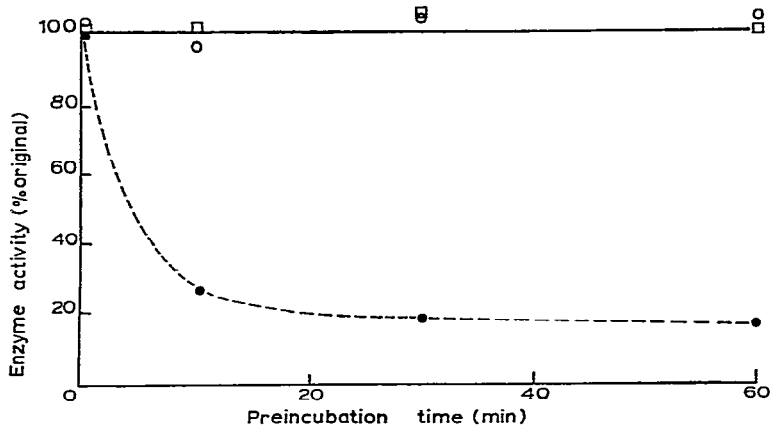

Fig. 1. Inhibition of *S. mutans* glucosyltransferase after different times of preincubation with periodate-oxidized dextran. Enzyme was preincubated with dextrans (170 μg/mL) at pH 6.0 and 37°, and activities were determined as described in Materials and Methods. Unoxidized dextran (○), 100%-oxidized dextran (□), and 10%-oxidized dextran (●).

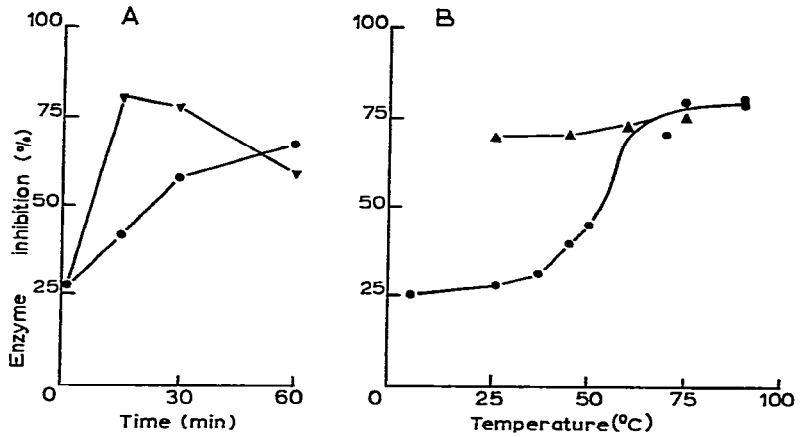

Fig. 2. Effect of storage and heat on the ability of 10%-oxidized dextran solutions to inhibit *S. mutans* glucosyltransferase. (A) Freshly prepared solutions (1 mg/mL) of unoxidized clinical dextran (not shown) and 10%-oxidized dextran were heated for the times indicated at 50 (●) or 90° (▼). Glucosyltransferase solution was preincubated with the treated dextran solutions for 30 min under the conditions described in the legend to Fig. 1, and the activity of the enzyme was assayed under standard conditions. (B) Freshly prepared solutions (1 mg/mL) of unoxidized clinical dextran (not shown) and 10%-oxidized dextran were stored for 7 days at 4 (●) and 26° (▲) and then heated for 15 min at the indicated temperatures. The abilities of the treated dextran solutions to inhibit glucosyltransferase was then determined as in (A). Enzyme inhibition by 10%-oxidized dextran is expressed as percent decrease in activity as compared with enzyme preincubated with similarly treated, unoxidized dextran.

7 days at 4° did not significantly increase its initial, low inhibition of enzyme activity, but storage at 26° caused a slow increase of the inhibitory capacity of the oxidized dextran, which reached a near-maximum (70% inhibition of enzyme activity) in 7 days. Subsequent incubation of this preparation for 15 min at higher temperatures

increased its inhibitory activity only slightly, but similar incubations of the oxidized-dextran solution previously stored at 4° resulted in a marked increase in its inhibitory capacity when the incubation temperature was 60° or higher (Fig. 2B). Incubation of the 10%-oxidized dextran for 60 min at 50° did not induce the maximum capacity to inhibit glucosyltransferase, whereas, at 90°, full inhibitory capacity was induced within 15 min (Fig. 2A). A longer incubation-period (60 min) at 90° caused partial loss of the inhibitory capacity of 10%-oxidized dextran.

Preincubation of glucosyltransferase with 10%-oxidized dextran at pH 7.0 and 8.0 resulted in greater extents of inhibition than did preincubation at pH 6.0 (Table III). At the higher pH values, enzyme activity was also significantly inhibited by 100%-oxidized dextran, indicating that a non-specific interaction occurs between the enzyme and the polyaldehyde. Therefore, in order to minimize non-specific inhibition, all preincubations were conducted routinely at pH 6.0.

Oxidized dextrans subsequently reduced with sodium borohydride did not inhibit glucosyltransferase, and there was no enzyme inhibition when unoxidized dextran was included with 10%-oxidized dextran in the preincubation mixture (Table IV). When unoxidized dextran was added to the enzyme mixture after the preincubation period, there was a slow recovery of enzyme activity (Fig. 3A), indicating that the interaction between oxidized dextran and enzyme was reversible, at least in part. Although unoxidized dextran is added to the enzyme-assay mixture, no recovery of activity was detected in the assay mixture when the reaction of inhibited enzyme was monitored during 60 min (Fig. 3B).

Once activated, the same sample of 10%-oxidized dextran gave reproducible inhibitions of the same enzyme preparation, and also caused about the same extent of inhibition of enzyme prepared from different cultures of the same organism. With

TABLE III

EFFECT OF pH ON INHIBITION OF *S. mutans* GLUCOSYLTRANSFERASE BY PERIODATE-OXIDIZED DEXTRAN

Extent of oxidation of dextran (%)	*Enzyme activity after preincubation with oxidized dextran*[a]					
	pH 6.0		*pH 7.0*		*pH 8.0*	
	[^{14}C]Glucose incorporated (c.p.m.)	*Inhibition*[b] *(%)*	*[^{14}C]Glucose incorporated (c.p.m.)*	*Inhibition*[b] *(%)*	*[^{14}C]Glucose incorporated (c.p.m.)*	*Inhibition*[b] *(%)*
0	1260	0	1370	9	1180	0
10	530	60	250	82	95	92
100	1050	17	620	55	350	86
No dextran	1360	0	1340	2	1180	0

[a]Enzyme was preincubated for 10 min at 37° and the indicated pH values with oxidized or unoxidized dextran (170 μg/mL). Activity was determined under standard conditions as described in Materials and Methods. [b]Inhibition is expressed as the percent decrease in activity as compared to enzyme preincubated with unoxidized dextran at the corresponding pH value.

TABLE IV

EFFECTS OF BOROHYDRIDE REDUCTION OR THE PRESENCE OF UNOXIDIZED DEXTRAN ON THE ABILITY OF OXIDIZED DEXTRAN TO INHIBIT THE GLUCOSYLTRANSFERASE OF *S. mutans*

Extent of oxidation of dextran (%)	*Activity of preincubated enzyme (c.p.m. [^{14}C]glucose incorporated)*			
	Oxidized dextran[a]		*Borohydride treatment*[b]	
	Alone	*Plus unoxidized dextran (670 μg/mL)*	*Untreated*	*Treated*
0	1040 (0)[c]	1000 (0)[c]	1180 (0)[c]	
5			350 (70)	1380
10	315 (70)	990 (1)	230 (81)	1300
100	930 (11)	910 (9)	1130 (4)	1115

[a]Enzyme was preincubated at pH 6.0 and 37° for 10 min with oxidized dextrans (170 μg/mL) in the presence or absence of unoxidized, clinical dextran (670 μg/mL). [b]Enzyme was preincubated for 30 min with oxidized or with borohydride-treated, oxidized dextrans (170 μg/mL). [c]Numbers in parentheses indicate the percent decrease in activity as compared to that of enzyme preincubated with unoxidized dextran under otherwise identical conditions.

one exception, crude glucosyltransferase-preparations from several strains of *S. mutans* were significantly inhibited by 10%-oxidized dextran, as was a glucosyltransferase preparation from *S. sanguis* 903–1600 (Table V). The extents of inhibition of many of the crude enzyme-preparations were similar to that of 400-fold purified glucosyltransferase of *S. mutans* 6715–49 (Table V). The enzyme that was not inhibited was obtained from mutant strain no. 33 of *S. mutans* 6715-13, which synthesizes only soluble dextran from sucrose. The glucosyltransferase of an isolate from the teeth of one of the authors (EES) also synthesized only soluble dextran, and was not inhibited by oxidized dextran.

Most of the glucosyltransferase in sucrose-fortified cultures of *S. mutans* 6715-49 is associated with an insoluble dextran–cell complex, and part of this activity may be "solubilized" by treating the complex with a solution of soluble dextran[15,25]. About 70% less enzyme activity was "solubilized" by 10%-oxidized dextran than by unoxidized dextran, but the amount of glucosyltransferase activity that remained associated with the insoluble dextran was the same (Table VI). This result indicates that 10%-oxidized dextran inhibits the soluble enzyme-component, but not the irreversibly bound, cell-associated, enzyme component.

The specificity of glucosyltransferase inhibition by oxidized dextran is shown in Table VII. Fructosyltransferases of two strains of *S. mutans*, and the phosphorylases of rabbit liver and potato, were not inhibited by oxidized dextran, under conditions in which oxidized dextran significantly inhibited glucosyltransferase. Oxidized glycogen and starch inhibited neither glucosyltransferase nor the phosphorylases for which these polysaccharides act as substrates.

In studies for which experimental details are not given, the effect of the inhibi-

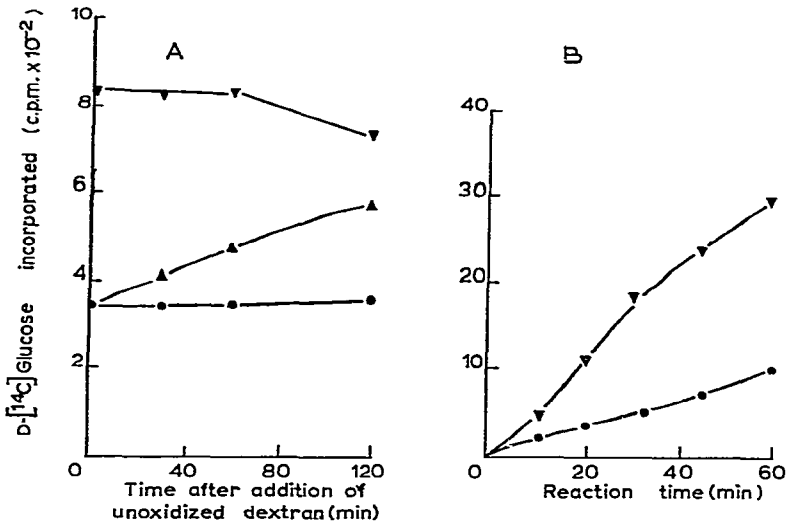

Fig. 3. (A) Partial recovery of enzyme activity on addition of unoxidized dextran, after preincubation of enzyme with 10%-oxidized dextran. Enzyme solution (100 μL) was incubated at 37° and pH 6.0 in mixtures (400 μL) containing sodium azide (20 μg) and 50mM sodium phosphate in the presence of 10%-oxidized dextran (100 μg) and/or unoxidized dextran (100 μg). After 30 min, unoxidized clinical dextran (100 μg in 0.1 mL) was added to one of two mixtures containing 10%-oxidized dextran (▲), and water (0.1 mL) was added to the other (●). Water (0.1 mL) was also added to the mixture containing oxidized and unoxidized dextran (▼), and to the mixture containing unoxidized dextran only (not shown). The mixtures were reincubated at 37° and sample were removed at the indicated times for enzyme assay as described in Materials and Methods. [^{14}C]Glucose incorporated in the assays of enzyme incubated in the mixture containing only unoxidized dextran (not shown) decreased from 1180 to 1020 c.p.m. during 60 min of reincubation. (B) Comparison of dextran synthesis under standard assay-conditions by glucosyltransferase preincubated with 10%-oxidized clinical dextran (●) or with unoxidized dextran (▼). Enzyme solution (25 μL) was preincubated at 37° and pH 6.0 in a number of mixtures (100 μL) containing 50mM sodium phosphate and 10%-oxidized dextran or unoxidized clinical dextran (25 μg). After 30 min, a solution (50 μL) containing [^{14}C]sucrose (0.025 μCi, 1 mg) and clinical dextran (25 μg) was added to each mixture and, at the indicated times, the amount of [^{14}C]glucose incorporated into dextran was determined.

tion of soluble enzyme by oxidized dextran, and the direct effect of oxidized dextran on the adherence of *S. mutans* cells, was examined by using modifications of the two assay-systems described by Mukasa and Slade[27] and by Kuramitsu[28]. Soluble glucosyltransferase, 70% inhibited by 10%-oxidized dextran, was observed to be equally as effective as the uninhibited enzyme in causing the adherence to glass of heat-killed cells suspended in sucrose solution. Also, the dextran coating formed on the side of a test-tube by the residual activity of the inhibited enzyme incubated with sucrose was as effective in adhering the dead cells as was the dextran coating formed by the uninhibited enzyme. In an assay system in which the adherence to glass of resting cells suspended in sucrose solution depends on the activity of the cell-bound glucosyltransferase, neither the presence of 10%-oxidized dextran nor of unoxidized dextran affected the extent of cell adherence.

An equal amount of soluble glucosyltransferase activity was elaborated by

TABLE V

INHIBITION BY 10%-OXIDIZED DEXTRAN OF GLUCOSYLTRANSFERASE PREPARATIONS FROM DIFFERENT MICROORGANISMS

Microorganism source of enzyme preparation[a]	*Enzyme activity after preincubation with oxidized or unoxidized dextran*[b] *(c.p.m. [14C]glucose incorporated)*		
	Unoxidized dextran	*10%-Oxidized dextran*	*Inhibition (%)*
S. mutans 6715-49			
Crude	800	250	69
Purified (400 fold)	400	132	67
S. mutans AHT	1510	560	63
BHT	640	315	51
K-IR	895	385	57
FA-I	2860	1040	64
GS-5	2350	855	64
S. mutans 6715-13			
Wild type	2505	775	69
Mutant no. 4	2000	1355	33
Mutant no. 33	350	365	0
S. mutans 903-1600	530	225	58

[a]All enzyme preparations were crude ammonium sulfate fractions obtained from cell-free culture media, except for the purified preparation from *S. mutans* 6715-49. [b]Enzyme solutions were preincubated for 30 min with 10%-oxidized or unoxidized dextran (170 μg/mL), and enzyme activity was determined under standard conditions.

TABLE VI

RESISTANCE OF CELL-ASSOCIATED GLUCOSYLTRANSFERASE TO INHIBITION BY OXIDIZED DEXTRAN

Cell treatment[a] *(extraction solution)*	*Glucosyltransferase activity (c.p.m. [14C]glucose incorporated)*					
	Cell-associated		*Soluble*		*Total*[b]	
	c.p.m.	%	*c.p.m.*	%	*c.p.m.*	%
Sodium phosphate (50 mM)	1050	98	25	2	1075	100
Unoxidized dextran (250 μg/mL)	560	52	620	58	1180	110
10%-Oxidized dextran (250 μg/mL)	590	55	180	17	770	72

[a]Cells of *S. mutans* 6715-49 were grown overnight in a culture medium containing 0.5% sucrose instead of 1% D-glucose. Cells were washed 3 times in 50mM sodium phosphate buffer (pH 6.0), and dispersed with vigorous agitation in one twentieth of the volume of the original culture. Portions of the cell suspension were incubated for 30 min at 37° in the presence of buffer, unoxidized dextran, or 10%-oxidized dextran as indicated, and then centrifuged. Enzyme activities in the supernatant solutions (soluble) and cells twice washed with buffer (cell-associated) were determined under standard assay conditions. [b]Total enzyme activity was calculated from the sum of cell-associated and soluble activities. Percent of the total activity recovered after treatment of cells with buffer was arbitrarily taken as 100.

TABLE VII

SPECIFICITY OF OXIDIZED DEXTRAN FOR INHIBITION OF GLUCOSYLTRANSFERASES

Enzyme source[a]	*Enzyme activity after preincubation with 10%-oxidized polysaccharides*[b]		
	Dextran	*Glycogen*	*Amylose*
Glucosyltransferase (*S. mutans* 6715-49)	40	93	119
Fructosyltransferase (*S. mutans* BHT)	100		
Fructosyltransferase (*S. mutans* FA-1)	107		
Glycogen phosphorylase *a* (rabbit liver)	98	89	83
Starch phosphorylase (potato)	98	102	93

[a]Glucosyltransferases and fructosyltransferases were crude, ammonium sulfate fractions obtained from the cell-free culture liquor of D-glucose-grown cells. Activities of glucosyltransferases were measured by the incorporation into dextran of [^{14}C]glucose from [^{14}C]sucrose([U-^{14}C]glucosyl). Activities of fructosyltransferases were measured by the incorporation of [^{3}H]fructose into fructan from [^{3}H]sucrose([1-^{3}H]fructosyl). [b]Enzyme activity of preincubated enzymes is expressed as percent of activity obtained after preincubation of the enzymes with the unoxidized polysaccharides.

cells of *S. mutans* 6715-49 grown for 12 h in D-glucose culture-media containing either unoxidized dextran or 10%-oxidized dextran (0.5–5 mg/mL). In experiments to account for the lack of enzyme inhibition under these culture conditions, we observed that a soluble glucosyltransferase-preparation was inhibited ~40% less by 10%-oxidized dextran when peptone–yeast-extract broth (a component of the cell-culture medium) was included in the preincubation mixture. This result suggested the possibility that the dialdehyde groups of the dextran derivative were interacting with peptides or amino acids in the broth, but when such purified proteins as crystalline bovine serum albumin or lysozyme (20 mg/mL) were substituted for the broth, the enzyme was not protected against inhibition by 10%-oxidized dextran.

In preliminary animal experiments, conducted in collaboration with Mrs. Dorothea B. Fitzgerald, Dental Research Unit, University of Miami School of Medicine, the addition of 10%-oxidized dextran (0.5–5 mg/mL) to the drinking water of hamsters maintained on a cariogenic diet did not significantly decrease the incidence of caries.

DISCUSSION

The inhibitory effect of 5- and 10%-oxidized clinical dextran (Table I), which occurs rapidly during preincubation with *S. mutans* glucosyltransferase (Fig. 1), probably involves the formation of a Schiff base or a carbinolamine derivative similar to that involved in the reaction wherein enzymic proteins are aggregated or immobilized by attachment to the dialdehyde functional-groups of periodate-oxidized glucans. As with solutions of "dialdehyde starch" used for enzyme immobilization[29], solutions of the oxidized-dextran inhibitor also required heat-treatment to increase

their reactivity (Fig. 2), presumably because high temperatures disrupt molecular associations that mask the reactive, dialdehyde groups. The structures and reactivities of dialdehydes obtained by periodate oxidation of carbohydrates have been reviewed[30]. The possibilities for formation of hemialdals by hydration of two aldehyde groups, or of hemiacetals by the interaction of one aldehyde group with a hydroxyl group, are greatly increased in oxidized polysaccharides because these reactions may be inter- as well as intra-molecular. The absence of carbonyl absorption in the i.r. or u.v. spectra of periodate-oxidized dextran has been assumed to be the result of hydration of dialdehyde groups[31], and the increasing difficulty we experienced in dissolving freeze-dried samples of oxidized dextrans as their degree of oxidation increased is consistent with the presence of intermolecular cross-links. Thus, 100%-oxidized dextran formed a stable gel in cold water that required extensive dilution at 70° to dissolve it.

Interaction between aldehyde groups of the oxidized dextran and the functional groups of the glucosyltransferase protein is indicated by the significant inhibition that occurs when the enzyme is preincubated with 100%-oxidized dextran at pH values >6.0 (Table III), and by the lack of inhibitory power at pH 6.0 (Table VI), and at pH 7.0 and 8.0 (data not shown), of all of the oxidized dextrans after their aldehyde groups had been reduced by borohydride. It is not known whether this interaction with the protein involves one or both of the functional groups of the dialdehyde formed by periodate oxidation of dextran.

The greater potency for enzyme inhibition of dextrans subjected to low degrees of oxidation (Table I) suggests that the affinity of glucosyltransferase for unmodified portions of the dextran structure is of critical importance for the specific inhibition of enzyme activity. Random oxidation of 5–10% of the glucose residues in clinical dextran apparently introduces a sufficient number of dialdehyde groups to allow efficient interaction with reactive groups on the enzyme, whilst leaving intact long sequences of unoxidized D-glucose residues that retain affinity for the enzyme, and bring aldehyde groups into close proximity to reacting groups near the glucosyltransferase binding-site. Successful competition for the same binding-site may explain how unoxidized dextran prevents enzyme inhibition by 10%-oxidized polysaccharide (Table IV) but, because it does not compete in the chemical interaction with groups at the binding site, the unoxidized glucan would not be expected to restore activity rapidly to enzyme previously inactivated by preincubation with 10%-oxidized dextran alone (Figs. 3A and 3B). The slow recovery of activity observed in the presence of clinical dextran (Fig. 3A) probably results from instability of the covalent linkages formed between glucosyltransferase and the aldehyde groups of oxidized dextran. Borohydride reduction of a 55%-inhibited enzyme prevented its reactivation in the presence of clinical dextran (data not shown), but this effect was complicated by the instability of the inhibited and uninhibited enzyme-samples after treatment with sodium borohydride.

Specificity of the inhibitory interaction of 10%-oxidized dextran is evident from its ability to inhibit almost all the glucosyltransferase preparations tested

(Table V), in contrast to its failure to inhibit fructosyltransferases from *S. mutans* (Table VII). Specificity also is revealed by the lack of inhibitory action of 10%-oxidized dextran against the phosphorylases of rabbit liver and potato, and by the failure of 10%-oxidized derivatives of the phosphorylase substrates, glycogen and amylose, to inhibit glucosyltransferase (Table VII).

We have no evidence that the inhibition by oxidized dextran is specific for any particular type of soluble glucosyltransferase. The two glucosyltransferases that were not inhibited by 10%-oxidized dextran synthesized only soluble dextran from sucrose, but several enzymes that were inhibited, including that of *S. sanguis*, also synthesized large amounts of soluble dextran. One glucosyltransferase preparation that was not inhibited (not shown in Table V) contained a low concentration of soluble polysaccharide (0.2 mg/mL), which protected the enzyme activity of other glucosyltransferases from inhibition by 10%-oxidized dextran. The presence of polysaccharide may have been the reason for the lack of inhibition of this enzyme preparation, but it is not known if polysaccharide also was present in the uninhibited enzyme-preparation from mutant strain no. 33 of *S. mutans* 6715-13 (Table V). The similar extents of inhibition of crude and purified glucosyltransferase-preparations from *S. mutans* 6715-49 (Table V) indicate that there is not a preferential inhibition of a single component of the crude, soluble, enzyme-mixture, but in no case was all of the enzyme activity inhibited by 10%-oxidized dextran. About 20% of the original activity remained uninhibited, even after extended preincubation with a large amount of inhibitor (Fig. 1, Table II). The residual activity could not be distinguished from the original enzyme-activity in tests of its ability to induce the adherence of *S. mutans* cells, and the reason for its resistance to inhibition remains to be determined.

Our results suggest that inhibition of glucosyltransferases of *S. mutans* by oxidized dextran resembles the specific inhibition of bovine galactosyltransferase by periodate-oxidized uridine 5′-diphosphate[32]. There is good evidence that galactosyltransferase is inhibited by formation of a Schiff base between the 6-amino group of a lysine residue near the active site of the enzyme and the dialdehyde in oxidized uridine 5′-diphosphate. Further studies are required, however, to establish whether or not the inhibition of the bacterial glucosyltransferases by oxidized dextran operates by the same mechanism.

Cell-bound activities, associated with D-glucose- and sucrose-grown cells of *S. mutans* 6715-49, are neither solubilized by high-molecular-weight clinical dextran nor do they use the dextran as an acceptor in their synthetic reactions[25]. To explain this result, it was suggested that steric hindrance prevents the formation of a specific enzyme–polysaccharide complex with bound enzyme. A similar explanation would account for the inability of 10%-oxidized dextran to inhibit cell-bound glucosyltransferase (Table VI) and for the inability of oxidized and unoxidized dextran to affect the adherence to glass of D-glucose-grown, resting cells.

Thus, although the 10%-oxidized dextran used in these studies is a potent and specific inhibitor of soluble glucosyltransferase, its high molecular weight (60,000–90,000) may make it ineffective as an inhibitor of dextran synthesis by

glucosyltransferase of bacterial plaque, and may explain why it did not decrease the incidence of caries in animal studies.

Oxidized derivatives of low-molecular-weight dextrans and oligosaccharides, therefore, are being tested as inhibitors of the *S. mutans* enzymes in an effort to develop a potent inhibitor small enough to penetrate the dense matrix of dental plaque and inhibit cell-associated, enzyme activity.

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