Streptococcus mutans Dextranucrase: Stimulation of Glucan Formation by Phosphoglycerides

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Received for publication 20 October 1977

Lysophosphatidylcholine (LPC) and other phosphoglycerides stimulated water-insoluble and water-soluble glucan production by the Streptococcus mutans 6715 dextranucrase (EC 2.4.1.5). LPC stimulated crude extracellular dextranucrase 1.7-fold, the water-insoluble glucan-producing α form of the enzyme 6.5-fold, the water-soluble glucan-producing β form of the enzyme 2.1-fold, and the cell-associated dextranucrase 2.0-fold. Kinetic studies demonstrated that LPC did not change the $K_m$ for sucrose of α or β but increased the maximum velocity of the enzymes. The $K_m$ for LPC of the α enzyme was $10^{-5}$ M. LPC from various sources and synthetic preparations of lauroyl-LPC, myristoyl-LPC, and palmitoyl-LPC all stimulated glucan formation. Portions of phosphoglyceride molecules including fatty acids, phosphatidic acid, glycerophosphoric acid, glycerophosphorylcholine, and choline, when tested individually or in combinations, did not enhance dextranucrase activity. The increased rates of glucan production caused by LPC and primer dextran were additive. Enzyme incubated with LPC before addition of sucrose was stimulated by dextran primer, and, conversely, enzyme treated with dextran was stimulated by addition of LPC with the sucrose substrate. Thus, dextranucrase can be activated by binding of intact phosphoglyceride molecules to a site on the enzyme that is distinct from either the glucosyl donor or glucosyl acceptor (primer) binding sites. Interactions between the S. mutans dextranucrase and amphipathic phosphoglycerides may explain properties of this enzyme which contribute to the cariogenicity of S. mutans.

Recent investigations have demonstrated that mammalian cell glycosyltransferase enzymes that are cell surface associated in their natural state may require the presence of a phospholipid for maximum catalytic activity (1, 15, 23, 31, 32, 40). However, there are few reports of bacterial glycosyltransferases that respond to the presence of phospholipids (8, 35, 36). Previous studies with the Streptococcus mutans 6715 dextranucrase (EC 2.4.1.5) have shown that this enzyme activity exists in cultures both extracellularly and firmly associated with the cell surface (18, 29, 42). In this communication we demonstrate that both the extracellular and cell-associated S. mutans dextranucrase activities can be markedly stimulated by phosphoglycerides and discuss the possible mechanism and significance of this effect for the functioning of this enzyme.

MATERIALS AND METHODS

Bacterium and growth conditions. S. mutans 6715 was obtained from Susan Michalek. Purification of dextranucrase from this strain by techniques previously presented (4, 17) revealed that it produced the various enzyme activities in ratios identical to those from the 6715 strain routinely used in these laboratories.

The cells used for studying the cell-associated dextranucrase were prepared as described previously (38), except that in one experiment the pH of the culture was maintained at 6.0 by periodic addition of 1 N sodium hydroxide.

Dextranucrase preparations. Most of the experiments presented in this report were performed with dextranucrase obtained from S. mutans 6715 by ammonium sulfate fractionation of cell-free culture supernatants and Bio-Gel column chromatography as described previously (17). The Bio-Gel enzyme preparation had a specific activity (17) of 3.1. Several studies were performed with the α and β forms of the enzyme, which were prepared by ion-exchange column chromatography and which produced water-insoluble and water-soluble glucans exclusively (17). The specific activities of the α and β preparations were 4.9 and 8.9, respectively.

Dextranucrase assays. The basic assay system for quantitating total alcohol-insoluble glucan production from $[^1^4]C$]sucrose has been described in detail (19). In the present study, activity was normally monitored by incubating 25 μl of diluted enzyme and 25 μl of test compound, organic solvent, or buffer (0.01 M sodium acetate, pH 5.5) at room temperature for...
10 min before initiation of the assay by addition of 75 μl of sucrose-containing reaction mixture. During incubation at 37°C, four 25-μl aliquots were removed at various times, placed on paper disks, and processed for quantitation of radioactive glucan as described previously (19). All assays were performed in the presence of T10 primer dextran at a concentration of 20 μM (16).

Separation by centrifugation and quantitation of water-soluble and water-insoluble glucans produced during dextranucrase assays were performed as described previously (17). The measurement of cell-associated dextranucrase activity was performed as described previously (38).

Other procedures. L-α-Lysophosphatidylcholine (LPC) preparations from egg yolk, bovine liver, and soybean and synthetically prepared LPC containing either lauric, myristic, or palmitic acid were obtained from Sigma Chemical Co., St. Louis, Mo. Before use, the phosphoglycerides were dissolved in 0.05 M sodium acetate buffer (pH 5.5) at a concentration of 5 mM.

L-α-Phosphatidylethanolamine (PE) made from egg lecithin (13 mg/ml of hexane-ethanol, 9:1), L-α-phosphatidylserine (PS) from bovine brain (7 mg/ml of benzene), and L-α-phosphatidylinositol (PI) from soybean (6 mg/ml of hexane-ethanol, 9:1) were obtained from Avanti Biochemicals, Inc., Birmingham, Ala. These phosphoglycerides were diluted to a concentration of 5 mM with 0.01 M sodium acetate buffer (pH 5.5) immediately before use.

Stearic acid, palmitic acid, myristic acid, and lauric acid (Nu-Chek-Prep, Inc., Elysian, Minn.) were dissolved in chloroform-methanol (49:1) at a final concentration of 15 mM. L-α-Phosphatidic acid, L-α-glycerophosphoric acid, and choline chloride (Sigma) were dissolved in the same solvent at a concentration of 15 mM. L-α-Glycerolphosphorylcholine (Sigma) obtained at a concentration of 5 mg/ml of methanol was dried under a stream of nitrogen and dissolved in chloroform-methanol (49:1) to a concentration of 15 mM immediately before use.

Protein was measured by the method of Lowry et al. (28), with bovine serum albumin as the standard.

RESULTS

Phosphoglycerides and glucan synthesis. Figure 1 illustrates the enhanced accumulation of alcohol-insoluble glucan when a Bio-Gel dextranucrase preparation was incubated in the presence of each of the phosphoglycerides LPC, PE, PS, and PI. When compared with the appropriate buffer or organic solvent controls, LPC appeared to be the most effective stimulator; after 60 min of incubation, this compound caused a 1.7-fold increase in the quantity of glucan present in the assay tube. The effect of the individual phosphoglycerides was not additive, since the addition of LPC to enzyme and either PE, PS, or PI did not cause greater glucan production from sucrose than each phosphoglyceride alone (data not shown).

When the effect of the various phosphoglycerides on water-soluble and water-insoluble glucan production by the Bio-Gel dextranucrase preparation was determined (Table 1), a differential effect on product formation was observed. Stimulation of water-insoluble glucan production was markedly enhanced by each of the phosphoglycerides. LPC caused a 2.6-fold increase in water-insoluble glucan synthesis, and PS caused a 1.4-fold increase. In contrast, LPC stimulated water-soluble glucan production only 1.1-fold, and PE had only a very small effect on synthesis of this type of product. PS and PI gave results comparable to those of PE in the production of water-soluble and water-insoluble glucan. The solubility of LPC in our sodium acetate buffer system and the effectiveness of this compound in enhancing glucan production caused us to choose this phosphoglyceride for additional studies.

LPC and different dextranucrase activities. Figure 2 illustrates that, after 60 min of

![Fig. 1. Effect of phosphoglycerides on time course of glucan production by S. mutans dextranucrase. Bio-Gel enzyme (20 μg of protein per ml) was assayed in the presence (●) and absence (○) of LPC (a), PE (b), PS (c), and PI (d). Phosphoglycerides were at a final concentration of 1 mM.](http://iai.asm.org/)
control (hexane-ethanol) controls (hexane-ethanol)

...tion of described diluting each LPC increased incubation.

In the study at this concentration, the rate of glucan synthesis by the purified α enzyme 6.5-fold and water-soluble glucan synthesis by the β enzyme 2.1-fold. These enhanced levels of stimulation of α and β, in comparison with the Bio-Gel enzyme, may reflect the less aggregated state of these forms of the enzyme (17). As indicated below (Fig. 3 and 4), the 1 mM concentration of LPC in this study was sufficient to give maximal stimulation of enzyme activity.

Table 2 presents results obtained when the effect of LPC on the cell-associated dextranu- crase activity of two different cell preparations was determined. At a concentration of 1 mM, the phosphoglyceride stimulated glucan accumulation 1.9-fold. Time course studies performed under similar conditions demonstrated that glucan synthesis by the cell-associated enzyme was directly related to incubation time for a minimum of 60 min (data not shown).

LPC and dextranu- crase kinetics. Figure 3 presents an analysis of the effect of LPC on the production of glucan at varying sucrose concentrations by the α and β forms of dextranu- crase. The Lineweaver-Burk plots demonstrate that the \( K_m \) for sucrose of either the α enzyme \( 2.2 \times 10^{-3} \) M or β enzyme \( 2.5 \times 10^{-3} \) M was unaltered in the presence of LPC, while the maximum velocity of each form of the enzyme was increased. With the α enzyme the maximum velocity was increased 3.6-fold, from 11.1 to 40.0 nmol/min per ml of enzyme. The increase in maximum velocity of the β enzyme was from 30.8 to 40.0 nmol/min per ml of enzyme.

Figure 4 presents a study in which the rate of synthesis of glucan from sucrose by the α form of dextranu-crase was determined in the presence of increasing concentrations of LPC. The dose response curve is sigmoidal, and near-maximal stimulation of enzyme was observed at an LPC concentration of 0.05 mM. Concentrations of LPC up to 1 mM showed no inhibition of enzyme activity. The \( K_m \) for LPC of the α enzyme was \( 10^{-5} \) M as determined by using the initial velocity obtained at the various LPC concentrations (Fig. 4, inset).

Comparison of LPC from various sources. The effect of various commercially available preparations of LPC on the Bio-Gel dextranu-crase was tested. LPC from egg yolk (containing primarily palmitic and stearic acid), bovine liver (containing primarily palmitic and stearic acid), and soybean (containing primarily C18 unsaturated fatty acids) all enhanced the rate of glucan accumulation. Synthetic LPC preparations containing exclusively lauric, myristic, or palmitic acid were also capable of stimulating glucan production to equivalent levels.

Portions of the LPC molecule. Various components of the LPC molecule were tested for stimulation of dextranu-crase in a solvent

<table>
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<tr>
<th>Table 1. Effect of phosphoglycerides on water-soluble and water-insoluble glucan synthesis by the S. mutans dextranu-crase</th>
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<tbody>
<tr>
<td>Conditions(^a)</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Control (buffer)</td>
</tr>
<tr>
<td>Plus LPC</td>
</tr>
<tr>
<td>Control (hexane-ethanol)</td>
</tr>
<tr>
<td>Plus PE</td>
</tr>
<tr>
<td>Control (benzene)</td>
</tr>
<tr>
<td>Plus PS</td>
</tr>
<tr>
<td>Control (hexane-ethanol)</td>
</tr>
<tr>
<td>Plus PI</td>
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</table>

\(^a\) Phosphoglycerides were at a final concentration of 1 mM. Organic solvent controls were prepared by diluting the appropriate solvent with buffer as for preparation of the 5 mM phosphoglyceride stocks as described in the text.

\(^b\) Bio-Gel enzyme was present at a final concentration of 20 μg of protein per ml, and the quantity of each type of glucan was determined after 60 min of incubation.

<table>
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<th>Table 2. Effect of LPC on cell-associated S. mutans dextranu-crase</th>
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<tbody>
<tr>
<td>Conditions(^a)</td>
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<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Expt 1</td>
</tr>
<tr>
<td>Expt 2</td>
</tr>
</tbody>
</table>

\(^a\) LPC was present at a final concentration of 1 mM.

\(^b\) Cell-associated glucan was determined after 60 min of incubation. The cells in experiment 2 were from a culture maintained at pH 6.0 during growth.
FIG. 3. Double-reciprocal Lineweaver-Burk plots of α (a) and β (b) dextranucrase activities measured in the presence (●) and absence (○) of LPC (1 mM) at various substrate concentrations. The α and β enzyme preparations were at 20 and 12 μg of protein per ml, respectively.

FIG. 4. LPC saturation kinetics of dextranucrase. Glucan production from sucrose by the α enzyme was assayed after 10 min of incubation in the presence of the indicated concentrations of LPC. Inset: Double-reciprocal plot of the same data; \( K_m = 10^{-5} \text{M} \).

system in which all of the compounds were soluble (Table 3). None of the compounds, individually or in appropriate combinations, caused an increase in enzyme activity. Intact LPC molecules were essential for stimulation of glucan production from sucrose.

Primer dextran and LPC. Figure 5 presents a study of dextranucrase stimulation by LPC in the presence and absence of primer dextran. In this experiment, LPC stimulated the enzyme 1.4-fold, dextran stimulated the enzyme 2.3-fold, and, when both LPC and primer were present, the enzyme activity was increased 3.6-fold. When present together, the primer and phosphoglyceride stimulated glucan production to a level greater than that obtained with either additive alone.

When dextranucrase was incubated with LPC at a concentration capable of giving maximum phosphoglyceride stimulation (1 mM) and then allowed to synthesize glucan from sucrose in the presence and absence of primer dextran, it was demonstrated (Fig. 6) that the enzyme responded to the presence of the primer. Conversely, when dextranucrase was incubated with excess primer and then allowed to make glucan from sucrose, it was shown (Fig. 7) that the enzyme responded to the presence of LPC. Since each of these preincubation studies were performed in the presence of excess dextran primer or LPC, it appears clear that stimulation of dextranucrase by these entities occurs through distinct, separable interactions with the enzyme.

**DISCUSSION**

Studies with a wide variety of enzyme systems

<table>
<thead>
<tr>
<th>Conditiona</th>
<th>Glucan formedb (nmol/μg of protein)</th>
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<tbody>
<tr>
<td>Control (chloroform-methanol)</td>
<td>40.9</td>
</tr>
<tr>
<td>Plus LPC</td>
<td>58.4</td>
</tr>
<tr>
<td>Plus stearic acid</td>
<td>40.9</td>
</tr>
<tr>
<td>Plus palmitic acid</td>
<td>40.7</td>
</tr>
<tr>
<td>Plus myristic acid</td>
<td>40.5</td>
</tr>
<tr>
<td>Plus lauric acid</td>
<td>39.3</td>
</tr>
<tr>
<td>Plus L-α-phosphatidic acid</td>
<td>33.0</td>
</tr>
<tr>
<td>Plus L-α-glycerolphosphate</td>
<td>41.6</td>
</tr>
<tr>
<td>Plus L-α-glycerophosphorylcholine</td>
<td>42.5</td>
</tr>
<tr>
<td>Plus choline</td>
<td>38.7</td>
</tr>
<tr>
<td>Plus stearic acid + L-α-glycerophosphorylcholine</td>
<td>42.1</td>
</tr>
<tr>
<td>Plus choline + L-α-phosphatidic acid</td>
<td>36.3</td>
</tr>
</tbody>
</table>

a Test compounds were at a final concentration of 1 mM. Chloroform-methanol (49:1) was at a final dilution of 1:15 in each tube.

b Bio-Gel enzyme was present at a final concentration of 20 μg of protein per ml, and total glucan was determined in triplicate after 60 min of incubation.
FIG. 5. **LPC stimulation of S. mutans dextran sucrase in the presence and absence of primer dextran.** Bio-Gel enzyme (20 µg of protein per ml) was assayed in the absence of LPC and dextran (△), in the presence of 1 mM LPC (△) or 20 µM dextran T10 (○), and with LPC plus dextran (●).

![Graph showing effect of LPC on dextran sucrase activity](image)

Fig. 5. Effect of incubation with LPC on dextran priming of the S. mutans dextran sucrase. Bio-Gel enzyme was incubated with LPC (1 mM) for 10 min before assay for sucrose-derived glucan formation in the absence (○) and presence (●) of dextran T10 (20 µM).

![Graph showing effect of dextran priming](image)

have demonstrated that lipid-enzyme interactions may have a profound modulating effect on catalytic function (6, 7, 10, 12). Addition of phosphoglycerides that are normal components of bacterial membranes (2, 7) to the S. mutans dextran sucrase causes stimulation of both watersoluble and water-insoluble glucan production from sucrose (Fig. 1, Table 1). LPC was shown to stimulate the various forms of the dextran sucrase (Fig. 2, Table 2), and intact LPC molecules appeared to be required for the effect (Table 3). Although activation of bacterial enzymes by lipids has been observed by many investigators (see reference 12), there are only a few reports of the effect of lipids on bacterial glyco-

...syltransferases (8, 35, 36). Our preliminary observations with the S. mutans dextran sucrase emphasize the importance of examining such interactions by bacterial enzymes that are found associated with the cell surface and/or excreted into the surrounding environment.

It appears that the interaction of dextran sucrase with phospholipid causes an elevation of the maximum velocity of the enzyme with no modification of its affinity for the sucrose substrate (Fig. 3). This observation is consistent with previous work with numerous lipid-activated enzymes in which effects of lipids on the \( K_m \) of the enzymes were sometimes observed and increases in maximum velocity were consistently demonstrated (12). This lack of effect of LPC on the affinity of dextran sucrase for sucrose and the observation that the phosphoglyceride does not alter the ability of the enzyme to be primed with dextran (Fig. 5 to 7) support the proposal that LPC interacts with dextran sucrase at a site distinct from either the glucosyl donor site or glucosyl acceptor site. These results support the findings of Burckhardt and Guggenheim (3), who presented data suggesting the presence of additional binding sites on the dextran sucrase that could interact with molecules capable of stimulating enzyme activity.

Detailed studies on the lipid-activated galactosyltransferase of Salmonella typhimurium (8, 35, 36) have demonstrated that the phospholipid component functions by interacting with the lipopolysaccharide acceptor rather than the enzyme. The stimulation of dextran sucrase by LPC in the absence of exogenous primer (Fig. 5) indicates that a similar mechanism is probably not working in our system. Although the possible presence of endogenous acceptor in the enzyme...

FIG. 7. **Effect of incubation with dextran on LPC stimulation of the S. mutans dextran sucrase.** Bio-Gel enzyme (20 µg of protein per ml) was incubated with dextran T10 (20 µM) for 10 min before assay for sucrose-derived glucan formation in the absence (○) and presence (●) of LPC (1 mM). Assays were performed in the presence (a) or absence (b) of additional primer dextran.
preparation might argue against this suggestion, studies with [14C]LPC have failed to show an interaction of phosphoglyceride with either primer dextran or product glucan during polysaccharide synthesis (Harlander and Schachtele, unpublished data).

The results obtained when the response of dextranase to increasing concentrations of LPC was studied (Fig. 4) are important for several reasons. First, the response to LPC was sigmoidal. This effect is typical of many lipid-activated enzymes and has been proposed to reflect stabilization of active protein conformation (10, 11, 12). Burckhardt and Guggenheim (3) have proposed that stimulation of dextranase results from formation of a more stable conformation (118:1-7). Ciardi, J. 1976. 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.

The high affinity of LPC for dextranase (Fig. 4) may be important for future attempts to determine the functioning of this enzyme in promoting the cariogenicity of S. mutans. Because the polar hydrophilic head and nonpolar hydrophobic hydrocarbon tail of phosphoglycerides give these molecules unique physical properties, the association of dextranase with phospholipids during or after excretion from the S. mutans cell could partially explain the highly aggregated state of the enzyme in culture fluids (13, 14, 20, 24, 25, 23), the multiple forms of the enzyme routinely found during purification (5, 17), the variations in the forms of the enzyme produced under different growth conditions (22, 39, 42), and the ability of partially purified enzyme to attach specifically to the S. mutans cell surface (41). In addition, the stimulation of dextranase by serum and oral secretions (3, 9, 14, 26, 27, 37) could involve interactions with the phospholipids known to occur in these fluids (21, 30, 34). Thus, it would appear that many of the observations previously made during studies on the S. mutans dextranase reflect a natural relationship between enzyme and phospholipid. The frequency and functional consequences of this type of interaction in mammalian and some bacterial enzyme systems and the results presented in this manuscript support this proposal.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant DE 03654 from the National Institute of Dental Research. We thank Woon-Lam S. Leung and Lee C. Ostrum for their excellent technical assistance.

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