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THE HYDROLYSIS OF SUCROSE BY DEXTRANSUCRASE*

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ABSTRACT

Dextransucrase was shown to catalyze the hydrolysis of sucrose. The hydrolytic activity was found to be directly correlatable with dextransucrase activity on poly-(acrylamide) disc-gel electrophoresis. In studies on the hydrolysis of sucrose and formation of dextran as a function of time and substrate concentration, the two activities were found to be competitive with each other. Competition was also observed between hydrolysis and the transfer of D-glucosyl groups to added acceptors. The results suggest that the three activities, namely, polymerization, D-glucosyl transfer, and hydrolysis, compete for a form of the enzyme that is common to all three reactions. It is proposed that this form may be a D-glucosylated derivative of the enzyme.

INTRODUCTION

Dextransucrase catalyzes the formation of dextran by utilizing sucrose as the D-glucosyl-donor substrate[1]. It is produced by a variety of micro-organisms, many of which are cariogenic[2,3]. Although the principal reaction appears to be the rapid polymerization of D-glucose to form dextran[4–6], the enzyme has also been shown to catalyze bimolecular reactions that involve the transfer of D-glucosyl groups to acceptor substrates[4–8]. We have initiated studies on these reactions in order to establish a clear understanding of the relationships between them. In the course of these studies, we have observed that the enzyme also catalyzes the hydrolysis of sucrose to D-glucose and D-fructose; this is similar to an earlier observation, by Goodman and co-workers[9], and is important in terms of the implications that it may have in regard to the mechanism of action of dextransucrase. Furthermore, it was realized that detailed knowledge of the hydrolytic reaction could have an impact on techniques employed in mechanistic studies. For example, it may be necessary to utilize rapid techniques for studies on a D-glucosylated intermediate of the enzyme if the rate of hydrolysis is high. A careful examination of the reaction has been conducted and the results are presented herein.

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RESULTS

Formation of D-*glucose by dextransucrase.* — Dextransucrase may be assayed spectrophotometrically by measuring the formation of D-fructose with a system, coupled to formation of NADPH, using hexokinase, phospho-D-glucose isomerase, and D-glucose 6-phosphate dehydrogenase[10]. Although this system actually detects both D-glucose and D-fructose, a specific measurement of D-glucose can be made by omission of the phospho-D-glucose isomerase. The results of both of these analyses for reactions catalyzed by dextransucrase are shown in Fig. 1. D-Glucose and D-fructose were formed simultaneously, in the ratio of $\sim 1:6$ in reaction mixtures that contained sucrose only. Under the conditions employed, <1 nmol of D-fructose 6-phosphate/min was isomerized, which precludes the possibility that the apparent formation of D-glucose was due to slow conversion of D-fructose 6-phosphate into D-glucose 6-phosphate. The data in Fig. 1 also demonstrate that formation of D-glucose from sucrose is diminished when an acceptor substrate such as dextran[6] is present.

Evaluation of contamination by invertase. — Because many oral streptococci produce invertase[11-13], it was possible that the preparation of dextransucrase employed in these studies had such a contaminant, which could have been responsible

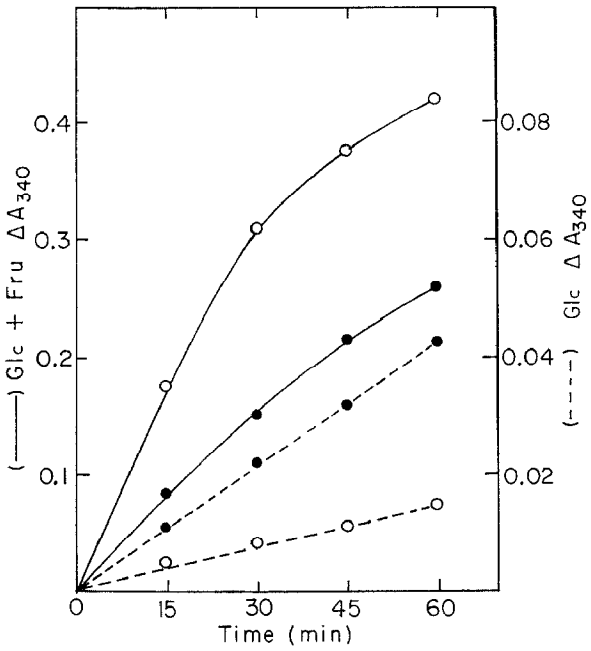

Fig. 1. Formation of D-glucose by dextransucrase (0.067 unit). [Dextransucrase was treated in the presence of 0.1M sucrose (—●—) alone or with 0.01M Dextran T-10 (—○—) in a volume of 200 μL. At 15, 30, 45, and 60 min, aliquots were withdrawn, and assayed for D-fructose plus D-glucose (——), which is the standard analysis for dextransucrase, or for D-glucose (- - -) by using the analysis described in Methods.]

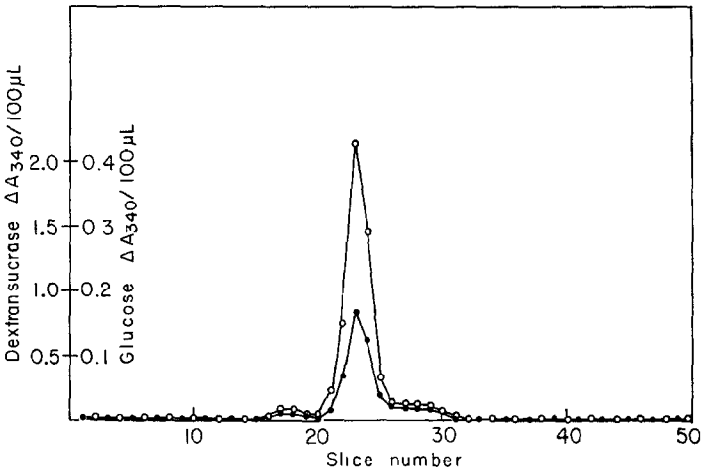

Fig. 2. Dextransucrase (1.0 unit) subjected to electrophoresis in the presence of 0.005% SDS and 0.01% Triton X-100, as described[14]. [The gel was sliced into 1-mm segments, and individually eluted in 0.1M sodium phosphate buffer, pH 6.0, overnight at 4°. Aliquots were removed from the eluates, and assayed for dextransucrase activity (—○—), and hydrolytic activity, by D-glucose formation (—●—).]

for the formation of D-glucose from sucrose. This question was examined by subjecting 1 unit of the enzyme to poly(acrylamide) disc-gel electrophoresis in the presence[14] of SDS and Triton X-100. Following electrophoresis, the gel was sliced, and each slice was eluted with 100 µL of 0.1M phosphate buffer, pH 6, overnight at 4°. Dextransucrase activity and sucrose-hydrolytic activity were measured in the eluates. The results, given in Fig. 2, showed that these two activities co-migrated on the gels. Identical results were obtained in another experiment, in which dextransucrase was localized *in situ* on one of a duplicate set of gels, and the second gel was eluted, and assayed for hydrolytic activity.

The utilization of raffinose, which is a typical substrate for invertases produced by oral bacteria[15], provided an alternative means of detecting such contamination. Dextransucrase is unable to utilize raffinose as a donor substrate[7]. When the enzyme (0.45 unit) was incubated with raffinose, no hydrolysis was observed. Under the conditions used, it would have been possible to detect 1×10^{-3} unit of invertase. We conclude from these experiments that there is no detectable invertase in the enzyme preparation, and that all of the hydrolytic activity observed is due to the action of dextransucrase.

Formation of D-*glucose as a function of time, and concentration of sucrose.* — We have observed that dextransucrase can be immobilized on hydroxylapatite. This form is fully active, and is convenient to use, as it can be rapidly removed from reaction mixtures by centrifugation or filtration. The hydrolysis of sucrose was examined in this way, using [^{14}C]sucrose as the substrate. As shown in Fig. 3, the supernatant fluids of such reaction mixtures contained sucrose, glucose, and fructose as determined by paper chromatography. The hydrolysis of sucrose may be assessed

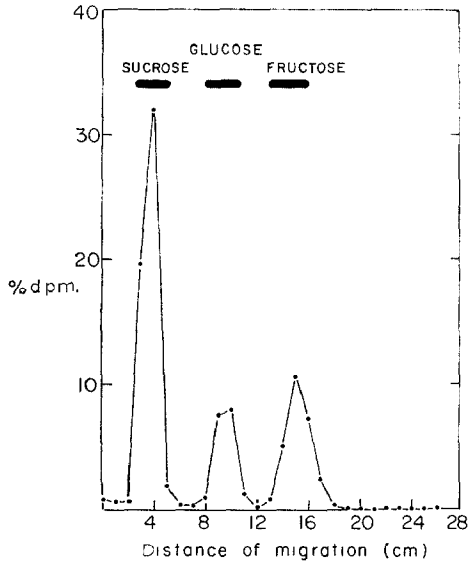

Fig. 3. Hydrolysis of radioactive sucrose. [Dextransucrase (0.445 unit), immobilized on hydroxylapatite, was treated with 0.2 mol of [U-^{14}C]sucrose (6 × 10^6 d.p.m.) in the presence of 2 μmol of Na_2HPO_4 buffer, pH 6.0, and 10 μg of SDS, in a total volume of 0.5 mL. After 1 min at room temperature, 5 mL of H_2O at 0° was added, and the mixture was immediately centrifuged. The supernatant fluid was removed, and an aliquot was spotted on Whatman No. 1 MM paper, chromatographed in solvent system *1*, and counted.]

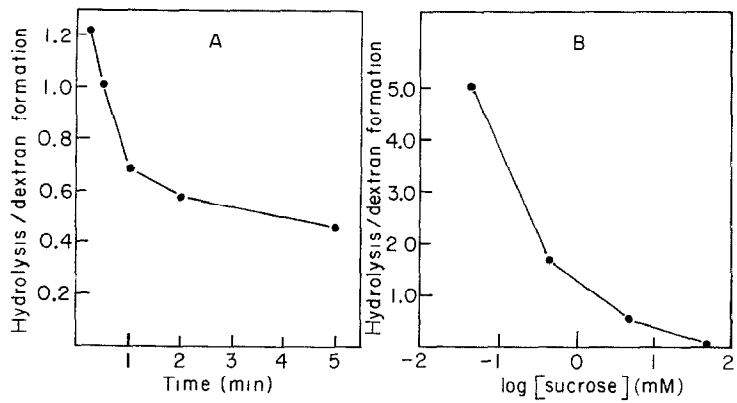

Fig. 4. A. The hydrolysis of sucrose as a function of time. [Immobilized dextransucrase (1.8 units) was incubated with 0.55 mol of [U-^{14}C]sucrose (60 × 10^6 d.p.m.) and 50 μmol of Na_2HPO_4 buffer, pH 6.0, in a total volume of 1 mL, at room temperature. Aliquots (200 μL) were transferred to 5 mL of H_2O at 0° at 0.25, 0.5, 1, 2, and 5 min. The mixtures were immediately centrifuged, and an aliquot of the supernatant fluid was chromatographed in solvent system *1*. The percentage of the total radioactivity that co-migrated with sucrose, fructose, and glucose was measured, and plotted as a function of time.] B. The hydrolysis of sucrose as a function of concentration. [Reaction mixtures containing immobilized enzyme (1.8 units), 0.05, 0.5, 5.0, and 50mM [U-^{14}C]sucrose (12 × 10^6 d.p.m.), and 10mM sodium phosphate, pH 6.0, were treated for 1 min at room temperature. Water (5 mL) at 0° was added to each reaction mixture, and the samples were analyzed as described in the legend to Fig. 4A.]

by the quantity of glucose formed. Dextransucrase activity is taken as the difference between the total radioactivity in fructose and that in glucose. In Fig. 4A, the ratio of hydrolysis to dextran formation is plotted as a function of time. At initial times, hydrolysis of sucrose is more rapid than formation of dextran. The ratio rapidly decreases to a point where, at 5 min, dextran formation is the dominant activity and occurs at about twice the rate of hydrolysis. The effect of the concentration of sucrose was evaluated by using the same system. Reactions were conducted over a range of 0.05 to 50mM sucrose. The ratio of hydrolysis to dextran formation was determined, and is plotted in Fig. 4B. As may be seen, the substrate concentration has a significant impact on the rates of the two reactions. At low concentrations, hydrolysis is the major reaction by a factor of 5. At high concentrations of sucrose, the formation of dextran is so rapid that it constitutes >99% of the products.

The results from these experiments may be interpreted in terms of competition between the two pathways. That is, under conditions that favor dextran formation, such as high concentration of sucrose, or at longer reaction-times, the fraction of sucrose undergoing hydrolysis is low.

DISCUSSION

The present results demonstrate the ability of dextransucrase to catalyze the hydrolysis of sucrose. This observation was originally made by Goodman *et al.*[9], who utilized a partially purified preparation of the enzyme. Several reports indicated that some bacteria that produce dextransucrase also produce an invertase[11-13]. Goodman *et al.*[9] attempted to inhibit, with silver nitrate, any possible contaminant; however, they did not provide a clear indication that the hydrolytic activity was due to dextransucrase. In our studies, we found that the enzyme preparation was not contaminated with invertase, as raffinose, which is a typical substrate for invertase, was not hydrolyzed; this implied that the hydrolytic activity observed was due to dextransucrase action, a conclusion clearly established by demonstration of the co-migration of hydrolytic activity and dextransucrase in poly(acrylamide) disc-gel electrophoresis. In addition, the observation that hydrolysis and polymerization are competitive supports this conclusion, as two different enzymes utilizing the same substrate would not behave in this way. It is probable that some of the invertase activity reported to be present in oral bacteria[11-13] can actually be attributed to dextransucrase action.

The hydrolysis of sucrose may be viewed as the transfer of the D-glucosyl group to water. The ability of the enzyme to catalyze this reaction may provide some insights into its mechanism of action. Such a transfer might occur in a direct manner[4], or it might involve the intermediacy of a D-glucosylated form of the enzyme, as proposed by Ebert and Schenk[16] and by Robyt *et al.*[17]. Our data (see Fig. 1) indicate that the rate of hydrolysis is diminished in the presence of an acceptor substrate, such as dextran[6]. It may be concluded that transfer to water and to an acceptor are competitive.

When the reaction is conducted in the absence of added acceptors, the principal reaction is *de novo* synthesis. The data shown in Figs. 4A and 4B indicate that, as the rate of *de novo* synthesis increases, hydrolysis decreases. This was observed as a function of time, which may be related to the production of sufficient dextran (either in terms of length, or concentration) to cause maximum rates of polymerization. It is known that dextran is an activator of dextransucrase[6]. The same relationship was observed as a function of concentration of sucrose, and this is consistent with the observation that the rate of formation of dextran increases over the range of sucrose concentrations used in these studies. It was therefore considered that hydrolysis is competitive with *de novo* synthesis.

In view of the observations reported, it may be concluded that the hydrolysis of sucrose is competitive both with *de novo* synthesis and with D-glucosyl transfer to an acceptor, and this competition may be regarded as additional evidence that a single enzyme catalyzes the three reactions. It would seem reasonable to speculate that the competition may be for a form of the enzyme that is common to all three activities, and this could be a D-glucosylated form of the enzyme.

The importance of recognizing the hydrolytic activity of dextransucrase can be illustrated by a comparison to sucrose phosphorylase[18], which catalyzes D-glucosyl transfer to phosphate, as well as to other acceptors, including water. In those studies, techniques had to be established to diminish hydrolysis before a D-glucosylated form of the enzyme could be demonstrated[19].

The data presented herein are consistent with the formation of a D-glucosylated intermediate that is involved both in D-glucosyl transfer and in polymerization, and that is also susceptible to hydrolysis.

EXPERIMENTAL

Materials. — Dextransucrase was purified by a modification of the procedure described by Huang *et al.*[10]. Radioactive [U-^{14}C]sucrose was purchased from New England Nuclear (Boston, Massachusetts). Hexokinase (Type F-300), phospho-D-glucose isomerase, D-glucose 6-phosphate dehydrogenase (Type XV), ATP, and $NADP^+$ were purchased from Sigma Chemical Company (St. Louis, Missouri). Hydroxylapatite was purchased from BioRad Laboratories (Richmond, California). Raffinose pentahydrate was obtained from Sigma Chemical Company, and purified by paper chromatography prior to use. Insta-Gel was purchased from Packard Instrument Company (Downers Grove, Illinois). All other reagents were reagent grade, and commercially available from common, chemical sources.

Enzymic analyses. — Dextransucrase activity was assayed[14] by a system that measures formation of D-fructose by coupling it to $NADP^+$ reduction, using 0.25 unit each of hexokinase, phospho-D-glucose isomerase, and D-glucose 6-phosphate dehydrogenase, a unit being defined as that amount of enzyme that catalyzes the formation of 1 μmol of D-fructose per min. Hydrolysis of sucrose was measured by the appearance of D-glucose, and utilized the same coupled system with the omission of

phospho-D-glucose isomerase. The level of phospho-D-glucose isomerase contaminating the other assay enzymes was measured. Conditions were established such that less than 0.001 unit of the isomerase was present in the D-glucose analysis.

Preparation of immobilized enzyme. — Dextransucrase was immobilized on hydroxylapatite. For an individual reaction, 3 mL of dialyzed enzyme (1.8 units) was stirred with 100 μL of hydroxylapatite, and the mixture was kept for 30 min at 4°, with occasional stirring. In this way, a minimum of 95% of the enzyme was adsorbed onto the hydroxylapatite. The samples were centrifuged, and after removal of the supernatant fluid, the immobilized enzyme was immediately used by resuspending it in a solution (100 μL) containing the substrate.

Chromatographic procedure. — Poly(acrylamide) disc-gel electrophoresis was conducted in the presence[14] of SDS–Triton X-100. These conditions result in disaggregated, enzyme forms that migrate and permit recovery of activity. The localization of activity on the gels was achieved *in situ* as described[14], or by measuring the dextransucrase activity and sucrose hydrolysis in eluates of 1-mm gel-slices.

Paper chromatography was performed on Whatman No. 1 MM paper, using 9:1:1 butanone–acetic acid–water saturated with boric acid (solvent system *1*) in the descending manner.

Radiological procedure. — All counting was done in a Packard 460 C liquid scintillation spectrometer. Radioactive chromatograms were cut into 1-cm strips and counted in 10 mL of scintillation fluid (15.2 g of PPO, and 380 mg of POPOP per 3.785 L of spectral-grade toluene). A standard curve for d.p.m. was prepared by counting a standard on Whatman No. 1 MM paper in the presence of increasing amounts of acetone as a quencher. Aqueous samples in 3 mL of water were counted in 5 mL of Insta-Gel.

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