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A D-GLUCOSYLATED FORM OF DEXTRANSUCRASE: PREPARATION AND CHARACTERISTICS*

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ABSTRACT

Dextransucrase was treated with [^{14}C]sucrose, and the product applied to gel-permeation columns. In the absence of the detergents SDS and Triton X-100, poor recovery of enzyme was observed; however, that enzyme which was recovered was labeled. In the presence of detergents, recovery was increased, but the material appeared to be a large aggregate (mol. wt. $>5 \times 10^6$). In addition, the ratio of D-glucose to enzyme suggested that a polymer had been formed. Disc-gel electrophoresis in the presence of a mixture of SDS and Triton X-100 showed similar results, and indicated that the aggregate was disrupted upon treatment with dextranase. Native enzyme that had been immobilized on hydroxylapatite could also be labeled with [^{14}C]sucrose, and the labeling followed saturation kinetics. The labeled protein could be released from the gel with 8M urea, but was aggregated. Radioactive sugars, free from protein, could be released by heating the labeled enzyme. The sugars released consisted of a mixture of D-glucose with oligosaccharides having an average chain-length of 17 D-glucosyl residues. The significance of these observations is discussed.

INTRODUCTION

Dextransucrase [EC 2.4.1.5] is a D-glucosyl transferase that catalyzes the transfer of the D-glucosyl group of sucrose to a growing chain of dextran[1]. Two models have been proposed for the pathway of dextran formation. One proposal suggests that new D-glucosyl groups are added at nonreducing termini[2], and evidence in support of this hypothesis has been obtained from the study of reactions in the presence of acceptors[3] or, in their absence, by using an analogous enzyme, amylosucrase[4]. The alternative proposal is based on the idea that chain growth oc-

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curs at the reducing end, while the growing chain is covalently attached to the enzyme[5,6]. In support of this concept, Robyt *et al.*[6] have shown, in a pulse-chase experiment with dextransucrase from *L. mesenteroides*, that new D-glucose residues are situated at the reducing terminus of the developing chain, and they suggested that two active sites are involved, one of which is occupied by a monomeric D-glucosyl residue, while the second has the growing dextranyl chain covalently attached. They proposed that the two sites alternate with each cycle of propagative steps.

The isolation of a D-glucosylated form of dextransucrase, and the demonstration that it is an intermediate in the reactions which it catalyzes, are central to an understanding of its mechanism. The data presented by Robyt *et al.* are consistent with a covalently bonded intermediate; however, direct isolation and investigations on its properties have not been reported. The studies reported employed impure enzyme, and the effect of contamination on the results is unknown. Furthermore, the experiments utilized enzyme immobilized on Bio-Gel P2 by chemical cross-linking, a procedure that resulted in a 99% loss of enzyme activity. It is unclear whether this loss was due to an alteration in the catalytic properties of all of the enzyme molecules, or to complete inactivation of 99% of the population.

We considered that reliable results could only be obtained in experiments that employed highly purified enzyme, and utilized procedures that resulted in little or no loss of enzyme activity. We have employed highly purified preparations of dextransucrase from *Streptococcus sanguis* ATCC 10558 in order to prepare a D-glucosylated form of the enzyme. In this and the accompanying paper[7], we report on the procedures for its preparation, and on its properties.

EXPERIMENTAL

Materials. — Dextransucrase was purified by a modification of the procedure described by Huang *et al.*[8]. The enzyme was homogeneous by the criteria of poly(acrylamide)-gel electrophoresis conducted in the presence[9] of SDS and Triton X-100. Multiple forms (usually two) of dextransucrase were present, and the specific activity ranged from 80 to 100 units/mg. Dextranase from *Penicillium* (Grade I) was purchased from Sigma Chemical Co. (St. Louis, MO) as were hexokinase, phosphoglucose isomerase, and glucose-6-phosphate dehydrogenase. Pyruvate dehydrogenase was a gift from Dr. P. A. Frey.

Dextran T-10, Sephadex G-75, Sepharose CL-6B 200, and Blue Dextran 2000 were purchased from Pharmacia Fine Chemicals (Piscataway, NJ); [^{14}C]sucrose, from New England Nuclear Corp. (Boston, MA); Insta-Gel, from Packard Inst. Co. (Downers Grove, IL); and hydroxylapatite and Biogel P-200, from Bio-Rad Laboratories (Richmond, CA).

Dextran was prepared enzymically from sucrose by using purified dextransucrase from *S. sanguis* 10558. The product was initially treated with Pronase, and precipitated in 50% ethanol. Precipitation was repeated 3 times, and a suspension

of the product in water was dialyzed for 2 d, and lyophilized. Dextran T-10 was also extensively dialyzed, and lyophilized prior to its use.

All other reagents were purchased from commercial sources.

Procedures. — Dextransucrase activity was measured as previously described[9]. A unit is defined as that amount of enzyme which catalyzes the formation of 1μmol of D-fructose per minute. Protein was measured either by the procedure of Lowry *et al.*[10] or Bradford[11].

Disc poly(acrylamide)-gel electrophoresis was conducted in the presence of SDS and Triton X-100 as described[9]. The gels were stained for protein by using Coomasie Brilliant Blue[9], and for enzyme activity by soaking the gels in sucrose and observing zones of opalescence where dextrans were formed[9]. Paper chromatography was performed in the descending manner in solvent system *I*, 6:1:3 (v/v) 1-propanol–ethyl acetate–H_2O, or system *II*, 9:1:1 (v/v) butanone–acetic acid–H_2O saturated with boric acid. Radioactivity measurements were made in a Model 460CD Packard Liquid Scintillation Spectrometer. Aqueous samples (3 mL) were counted in Insta-Gel (5 mL). Paper chromatograms were cut into 1-cm strips and counted in scintillation fluid (10 mL) consisting of PPO (15.2 g) and POPOP (380 mg) per 3.785 L (1 gallon) of spectral-grade toluene.

RESULTS

Preparation of D-*glucosylated enzyme: isolation by gel permeation.* — Initial experiments, designed to permit isolation of a D-glucosylated form of dextransucrase, utilized gel permeation as a means of separation. Fig. 1A shows the results obtained when native enzyme was pulsed with [^{14}C]sucrose for 30 s, immediately diluted with cold water, and applied to a column of Bio-Gel P-200 maintained at 4°. The enzyme was eluted in two peaks, one at the position of the native enzyme, and the second (major) peak at the exclusion volume. The shift in the elution volume of the major peak suggested that a change in molecular weight had occurred. Radioactivity was associated with this peak, and, based on a molecular weight[12] of 155,000, the ratio of D-glucose to enzyme molecule was estimated to be 120:1. It must be noted that only 3% of the enzyme activity that had been applied to the column could be recovered. Both the radioactivity and the enzyme activity were found to be associated with material at the top of the column. This behavior was similar to that encountered in earlier work, which showed that aggregate forms of the enzyme are filtered out at the top of poly(acrylamide) gel-filtration columns.

Similar experiments were performed in which the enzyme was subjected to dissociating conditions using[9] SDS and Triton X-100 prior to the [^{14}C]sucrose pulse. Following the reaction with sucrose, the enzyme was chromatographed on Sepharose 6B in the presence of the detergents. The results (see Fig. 1 B) indicated that the pulsed enzyme was in an aggregated state, whereas the native enzyme was not. The recovery of the pulsed enzyme was 109% with respect to activity, and

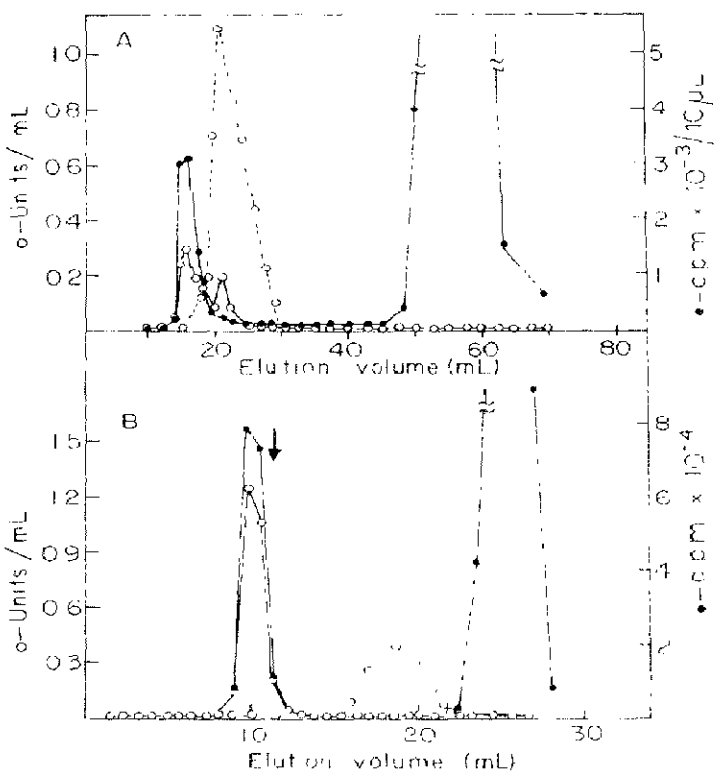

Fig. 1. Gel filtration of dextransucrase. [A. *P-200*. Native enzyme (38 units) was applied to a column (1.5 × 33.5 cm) of Bio-Gel P-200, and eluted with 0.01M phosphate buffer, pH 6.0 (○——○). A sample of the eluted enzyme (12.5 units) was incubated with 1 μmol of [^{14}C]sucrose (5 × 10^{5} c.p.m.), and 1 μmol of phosphate buffer, pH 6.0, in a total volume of 100 μL. After 30 s, H_2O (0.4 mL) at 4° was added, and the mixture was immediately applied to the same column of Bio-Gel P-200 and eluted as just described. Radioactivity (●——●) and enzyme activity (○——○) were measured. B. *Sepharose 6B-CL with SDS/Triton X-100*. Separate samples of dextransucrase (2.33 units) were mixed with 55 mg each of SDS and Triton X-100 in the presence of 1.1 mL of 0.1M sodium phosphate buffer, pH 6.0, and allowed to stand for 1 h at room temperature. One sample (○---○) was applied directly to a column (1.1 × 24 cm) of Sepharose 6B-CL, and the column was eluted at 4° with 0.1M sodium phosphate buffer, containing 0.005% SDS and 0.005% Triton X-100. The second sample (●——●) was added to [^{14}C-U]sucrose (1.1 μmol, 1.1 × 10^{6} d.p.m.) in a final volume of 1.0 mL. The mixture was immediately applied to the same column, and eluted in the same way. Enzyme activity (○) and radioactivity (●) were measured. Peak fractions were pooled, and total recovery of activity and of protein were determined. The arrow indicates the elution volume of pyruvate dehydrogenase complex[15] (mol. wt. 4.8 × 10^{6}).]

120% with respect to protein. The molar ratio of D-glucose to enzyme was estimated to be 192 μmol of D-glucose per μmol of enzyme.

Enzyme that had been treated with sucrose was also subjected to disc-gel electrophoresis under disaggregating conditions in the presence[9] of SDS and Triton X-100. The gels (see Fig. 2) showed that, with increasing concentrations of sucrose (gels A–D), there is aggregation, even in the presence of detergents. However, when the aggregate form is treated with dextranase (gel E), an electrophoretic pattern similar to, but not identical with, that for the native enzyme (gels A and G) is

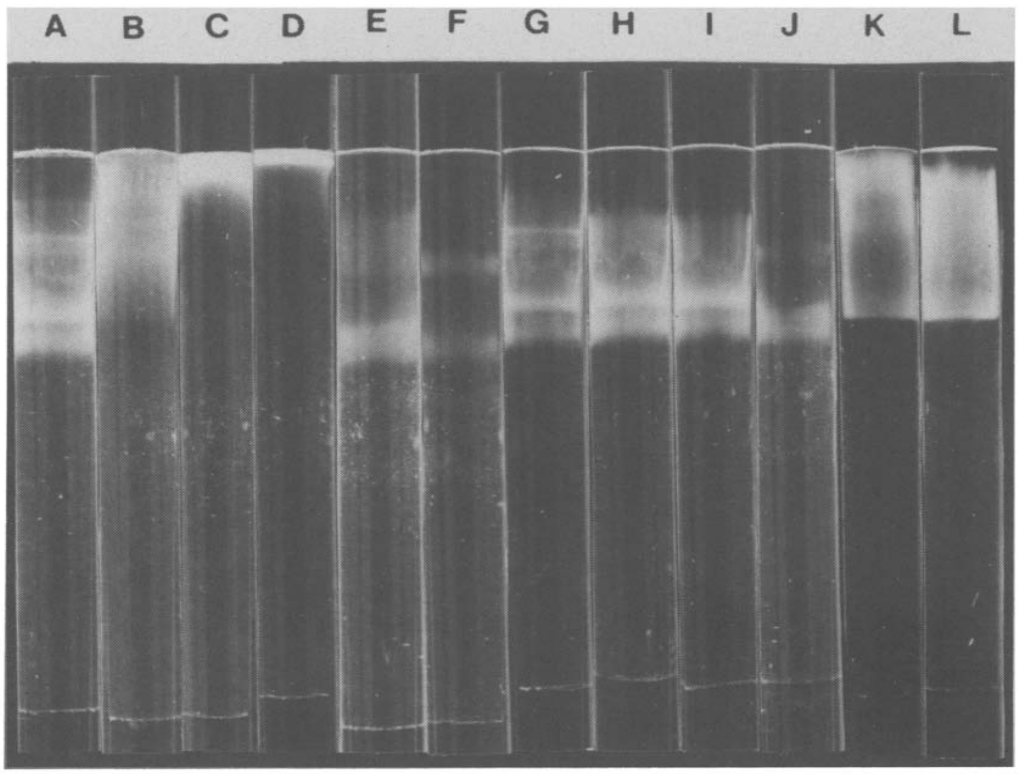

Fig. 2. Disc-gel electrophoresis of sucrose-pulsed enzyme. [Dextransucrase (0.31 unit, 3.7 μg of protein) was incubated for 15 min at 25° in the presence of 0.005% SDS, 0.01% Triton X-100, and 2mM sodium phosphate buffer, pH 6.0, in a final volume of 0.5 mL, with the following additions: (A) none; (B) 0.01mM sucrose; (C) 0.05mM sucrose; (D) 0.1mM sucrose; (E) 0.1mM sucrose, followed by 10 units of dextranase for an additional 15 min, (F) 10 units of dextranase; (G) none; (H) 0.1 mg of Dextran T-10/mL, (I) 1 mg of Dextran T-10/mL; (J) 1 mg of Dextran T-10/mL, followed by 10 units of dextranase for an additional 15 min; (K) 0.1 mg of dextran from *S. sanguis* 10558/mL; (L) 1 mg of dextran from *S. sanguis* 10558/mL. At the end of the incubations, glycerol (1 drop) and 0.05% Bromophenol Blue (1 μL) were added to the solutions, and the samples were applied to 5% poly(acrylamide) gels, subjected to electrophoresis, and stained as described in Methods.]

observed. The pattern observed in E is also seen when native enzyme is treated with dextranase (gel F).

As controls for this series of experiments, the mobility of dextransucrase in the presence of added dextrans was evaluated. The electrophoretic behavior of the enzyme after exposure to two concentrations of Dextran T-10 (gels H and I), which is primarily a linear dextran, is virtually the same as that of the native enzyme. This means that aggregation did not occur, and that the enzyme does not form a tight association with this polymer. A similar study with dextran from *S. sanguis* 10558 (gels K and L) showed that the enzyme was distributed over a broad segment of the gels, suggesting that the enzyme binds more tightly to this dextran than to Dextran T-10. However, the electrophoretic pattern was entirely different from that observed with sucrose (gels C and D), as all of the enzyme had moved from the origin.

TABLE I

IMMOBILIZATION OF DEXTRANSUCRASE[a]

Sample	Enzyme + hydroxylapatite		Supernatant fluid		Pellet		Supernatant fluid		Pellet	
	Activity (U)	Recovery (%)	Activity (U)	Recovery (%)	Activity (U)	Recovery (%)	Activity (U)	Recovery (%)	Activity (U)	Recovery (%)
IX-B	0.742	100	0.00	—	0.644	87	0.507	68	0.104	14
			0.014	1.9	0.663	89	0.507	68	0.127	17
XI-A	0.520	100	0.2	0.001	0.403	78	0.299	58	0.110	21
			0.003	0.6	0.403	78	0.315	61	0.110	21
XV-D	0.624	100	0.00	—	0.604	97	0.533	85	0.078	13
			0.00	—	0.624	100	0.543	87	0.088	14
XVII-B	0.632	100	0.00	—	0.509	80	0.418	66	0.068	11
	0.684	100	0.008	1.0	0.526	77	0.416	61	0.091	13
			0.00	–	0.540	79	0.436	64	0.084	12
	0.636	100	0.00	—	0.486	77				
			0.00	—	0.499	79				
			0.00	—	0.493	78				
			0.00	—	0.506	80				

[a] Samples of dextransucrase (~0.65 unit) from four different preparations of enzyme were individually mixed with hydroxylapatite (25 mg) in the presence of Triton X-100 (500 μg ml⁻¹). The mixtures were then centrifuged and the supernatant fluids removed. The pellets were resuspended in 1 mM phosphate buffer, pH 6 (1 mL, 25 mg of hydroxylapatite) containing Triton X-100 (50 μg ml⁻¹), and enzyme activity was measured both in the supernatant fluids and the pellets. The enzyme was eluted by resuspending the pellets in 1 mL of 0.5M phosphate buffer, pH 6, containing Triton X-100 (50 μg ml⁻¹), following centrifugation, the activity was measured in the supernatant fluids and the residual pellets.

The results of these studies indicated that multiple residues of D-glucose became bound to the enzyme during a brief exposure to sucrose, and that this form of the enzyme was completely immobile. However, mobility was not lost upon exposure to preformed dextrans, which indicates that a unique association is formed between the enzyme and newly synthesized polymer. The aggregate formed by exposure to sucrose could not be dissociated under conditions known to dissociate aggregate forms of the native enzyme.

Preparation with immobilized enzyme. — The focus of these studies was preparation of a D-glucosylated enzyme in a form that could be utilized in studies of its characteristics. The fact that the D-glucosylated enzyme isolated by gel-permeation techniques was extensively aggregated meant that it could not be used

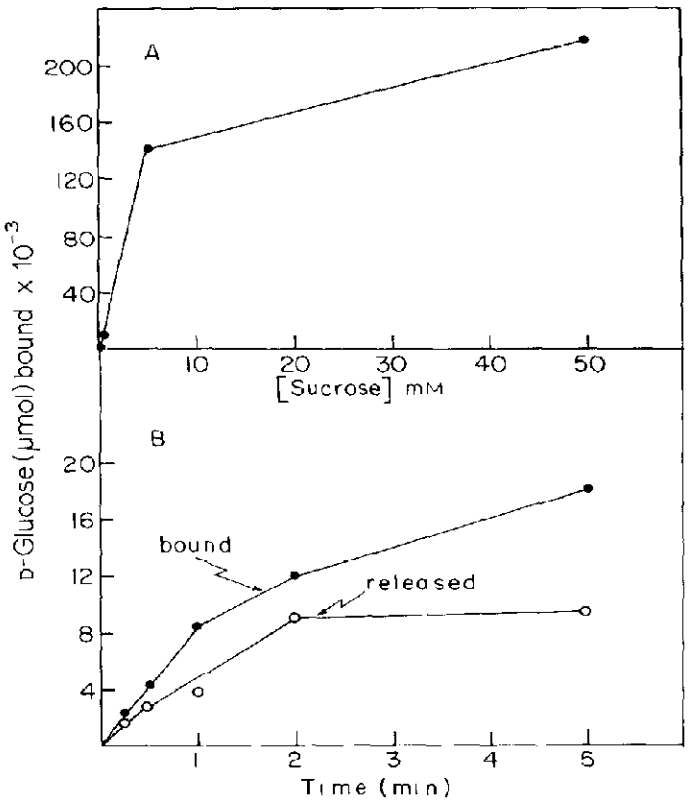

Fig. 3. Binding of D-glucose to immobilized enzyme. [A. *As a function of sucrose concentration.* Immobilized dextransucrase (1.8 units) was included with [^{14}C-U]sucrose (12×10^6 d.p.m.) at concentrations of 0.05, 0.5, 5.0 and 50mM in the presence of 10mM sodium phosphate buffer, pH 6.0, in a final volume of 0.5 mL. After 1 min at room temperature, H_2O (5 mL) at 4° was added to each mixture, and each was immediately centrifuged. The pellet was washed twice with H_2O (5 mL), and resuspended in H_2O (0.5 mL). The amount of D-glucose bound to the pellets was determined by liquid scintillation isotopic counting of 50-μL aliquots of the suspension. B. *As a function of time.* Immobilized enzyme (1.8 units) was incubated with [^{14}C-U]sucrose (60×10^6 d.p.m.) at a concentration of 0.55mM, in the presence of 50mM sodium phosphate buffer, pH 6.0, in a total volume of 1.0 mL at room temperature. Aliquots (200 μL) were transfered to H_2O (5 mL) at 4° at 0.25, 0.5, 1.0, 2.0 and 5.0 min. The mixtures were immediately centrifuged, washed as described for 3A,and counted for radioactivity (—○—) as described for 3A. The resuspended pellets were heated for 10 min at 93°, and centrifuged. The amount of glucose released (—●—) was measured by counting 50-μL aliquots of the supernatant fluids.]

for these types of studies. In order to avoid aggregate formation, the use of immobilized enzyme was examined. In such a system, enzyme molecules would be fixed to a matrix in a dispersed manner, which would prevent aggregation. In addition, such a system would permit rapid removal of substrates, either by centrifugation or filtration.

Dextransucrase has been immobilized on hydroxylapatite, and shown to remain active[13]. Before such an immobilized preparation could be employed for the intended studies, it was imperative to determine the level of activity that could be expressed in the bound form. Table I describes a series of experiments in which the recovery of activity in the immobilized state was measured. Following mixing with hydroxylapatite for 30 min, and centrifugation, the activity in the supernatant fluid and in the pellet were measured. Little if any activity was observed in the supernatant fluids, whereas between 77 and 100% of the original activity could be detected in the pellet, indicating that the enzyme is highly active in the bound state. In addition, it appears that a loss of activity resulted from the immobilization procedure. This may be seen in the last two columns of Table I, which describe the enzyme activity in the supernatant fluids and resuspended pellets obtained after elution of dextransucrase with 0.5M phosphate buffer. The sum of the activities in the two fractions is, within experimental error, equal to that observed in the immobilized enzyme. These data indicated that dextransucrase immobilized on hydroxylapatite might be employed for the preparation of a D-glucosylated form, which would be in a disaggregated state.

In order to explore this possibility, enzyme was immobilized on hydroxylapatite as described in Table I, and then treated with [^{14}C]sucrose at room temperature. At the end of the reaction time, phosphate buffer (5 mL) at 4° was added, and the mixture was centrifuged. The pellet was washed twice with H_2O (5 mL) at 4°, and resuspended in H_2O (0.5 mL). The data shown in Fig. 3A indicated that, as the concentration of sucrose is increased, the amount of isotope bound to the hydroxylapatite reaches a maximum, and appears to follow saturation kinetics. Fig. 3B shows the binding of the radio label as a function of time.

Two techniques were found to be effective in removing the labeled material from the support. In the presence of 8M urea buffered at pH 6.0 with 0.01M phosphate buffer, 53% of the enzyme activity, and 70% of both the protein and the isotope could be recovered in the supernatant fluid. When the released material was applied to a column of Sepharose 6B, an elution pattern similar to that in Fig. 1B was observed; this indicated that the enzyme had aggregated when it was released from the hydroxylapatite. Fig. 3B shows that a major portion of the bound isotope can be released by heating for 10 min at 93°; however, only 0.6% of the protein was detected in the supernatant fluids, leading to the conclusion that the isotope had been released from the protein. The material released by heating was characterized in several experiments. A sample was chromatographed on Sephadex G-75 (see Fig. 4), in order to estimate the size of the material. Two peaks were observed, both of which were included in the column matrix. The first peak

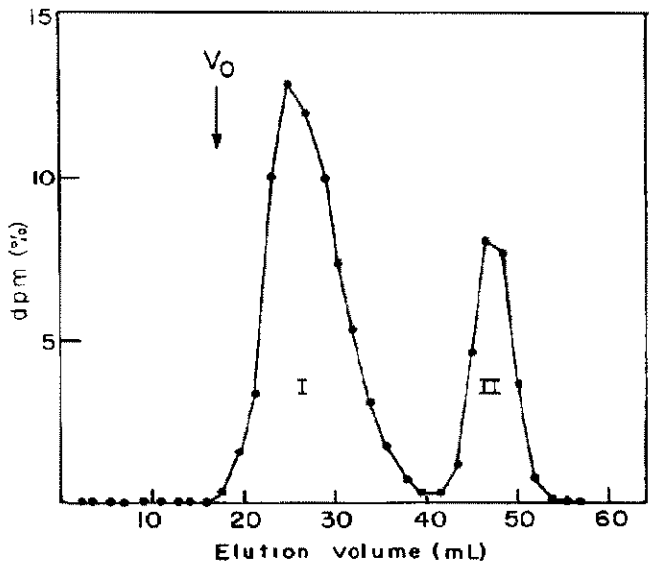

Fig. 4. Gel-filtration of heat-released material. [The heat-released material (see Fig. 3B), 17,000 d.p.m., was applied to a column (1.1 × 46.3 cm) of Sephadex G-75 that had been pre-equilibrated with 0.1M potassium phosphate buffer, pH 6.0, and eluted with the same buffer. Aliquots (100 μL) were counted as described in Methods.]

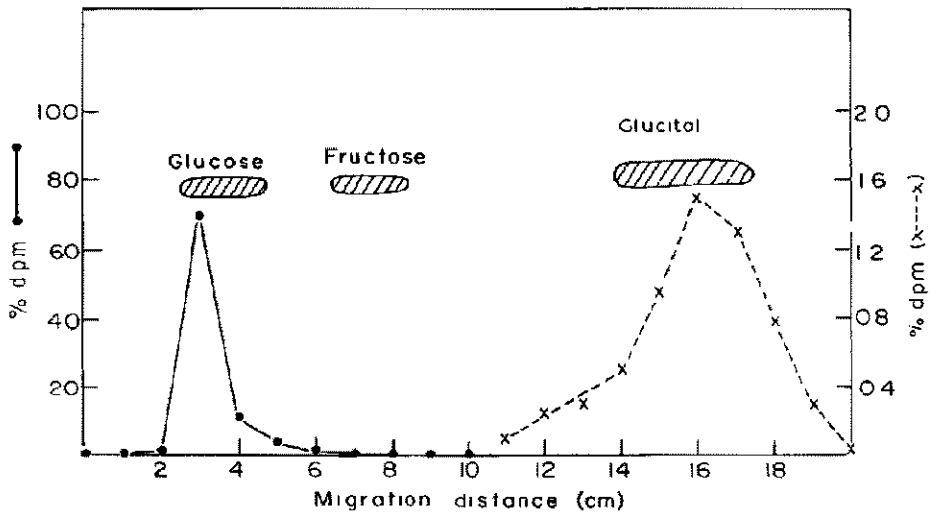

Fig. 5. End-group analysis of peak I. [The material in peak I (see Fig. 4) was pooled, and evaporated to dryness. Sodium borohydride (0.01M; 100 μL, pH 12) was added, and the mixture was kept for 15 h at 50°, made neutral, evaporated to dryness, and subjected to hydrolysis in M HCl (0.5 mL) for 5 h at 93°. The sample was evaporated to dryness, resuspended in H_2O (100 μL), and analyzed by paper chromatography on Whatman No. 1 MM paper in solvent *II*. The chromatogram was counted as described.]

(I), which accounts for 73% of the applied radioactivity, was eluted at a position consistent with its being an oligomer. The second peak (II), which constituted 26% of the isotope, was eluted at a position that corresponded to monosaccharides. Paper-chromatographic analysis of peak II in solvent *I* indicated that it was entirely D-glucose, whereas peak I material was non-mobile.

TABLE II

END-GROUP ANALYSIS OF HEAT-RELEASABLE MATERIAL[a]

Sample	*Origin (pmol)*	*Glucose (pmol)*	*Glucitol (pmol)*
1 Heat released	53.2	16.0	0.0
2 Reduced	51.4	0.4	15.7
3 Reduced, hydrolyzed	0.5	50.0[b]	17.2

[a]Material released by heating the pellets (69 pmol, 173,000 d.p.m.) was delivered to each of 3 tubes, and evaporated to dryness. Sodium borohydride (0.1 M; 0.1 mL, pH 12) was delivered to tubes 2 and 3, and allowed to react for 15 h at 50°. The solutions were made neutral, and evaporated to dryness. M HCl (0.5 mL) was added to tube 3, and this was heated for 5 h at 95°, cooled, and evaporated to dryness. Each sample was dissolved in H_2O (100 mL), and chromatographed on Whatman No. 1 MM paper, with solvent system *H*. The chromatograms were counted as described in Methods. [b]A small amount of an anhydro-D-glucose was formed during hydrolysis, and is included in the value for glucose.

The material in peak I was subjected to end-group analysis. A sample was reduced with sodium borohydride, followed by acid hydrolysis. The products were analyzed by paper chromatography as shown in Fig. 5. Only glucose and glucitol were observed, and they respectively accounted for 94.3 and 5.7% of the total isotope. From these data, the average chain-length was obtained by using the following expression.

$$\frac{\text{glucose (\%)} + \text{glucitol (\%)}}{\text{glucitol (\%)}} = \frac{100\%}{5.7\%} = 17.5$$

In a similar experiment, the entire material released by heating was subjected to end-group analysis. Samples were chromatographed as described in Table II, before (line 1) and after reduction with $NaBH_4$ (line 2), and after subsequent acid hydrolysis (line 3). The increase in glucitol observed after hydrolysis is taken to represent that formed from the reducing termini of the oligomers; using this value, an average chain length of 34 was obtained. The data obtained in this study may not be as accurate as those from the experiment described in Fig. 5, as the number of end groups was determined by the difference between two large numbers. However, the two sets of data are in general agreement, and indicate that an oligomer of D-glucose had been formed and was present on the enzyme.

It may also be seen from this experiment that there are fewer oligosaccharide chains than monosaccharide residues [by comparing the glucitol formed after reduction (line 2) with that derived from the reducing termini of oligomers (line 3 minus line 2)].

DISCUSSION

Interpretation of the results of experiments on the mechanism by which

dextransucrase catalyzes the formation of polymers has been unclear[6]. It was suggested that, during the course of the reaction, the enzyme becomes D-glucosylated[5,6]; however, the experiments supporting this premise employed enzyme that had lost 99% of its activity. The direct isolation of this form of the enzyme, and an examination of its properties, were not reported.

The present article describes experiments in which enzyme exposed to [^{14}C]sucrose was isolated by gel filtration on two types of support (Bio-Gel P-200 and Sepharose-6-B). In each case, the enzyme formed a large aggregate under conditions known to disrupt aggregates of the native enzyme. The data also showed that the enzyme had become heavily D-glucosylated, because between 120 and 193 D-glucose residues per enzyme molecule were found to be tightly bonded to the protein. The same properties were noted when sucrose-pulsed enzyme was examined by poly(acrylamide)-disc gel electrophoresis. The aggregation observed appears to be due to interactions between protein and dextran chains, as digestion with dextranase disrupts the aggregate. However, the complex-formation cannot be due to simple interaction between enzyme and dextran molecules, a conclusion drawn from experiments in which enzyme mixed with Dextran T-10, or the native dextran isolated from *S. sanguis* 10558, was subjected to electrophoresis. In both instances, the electrophoretic pattern was entirely different from that of the sucrose-pulsed enzyme, which remained entirely at the origin.

These observations suggest that the nature of the bonding in the complex formed during reaction with sucrose is quite different from that formed between the enzyme and preformed polysaccharide chains. It is possible that dextran chains, formed during the pulse with sucrose, are covalently bonded to the enzyme, as suggested by Robyt *et al.*[6]. It is also possible that growing polysaccharide chains bind to other enzyme molecules in a non-covalent manner; this type of interaction would provide for a more stable complex than one that is entirely noncovalent, as is that between the enzyme and exogenously added dextran. The fact that the ratio of D-glucose to enzyme is very high supports the concept that a polymer is formed on the enzyme. Polymer formation was established when the products bound to immobilized enzyme during a sucrose pulse were examined and found to contain oligosaccharides of moderate length.

It was considered undesirable to use aggregated enzyme for mechanistic studies, as it is important to know that all sites are equally accessible to substrate, and catalytically competent. Thus, for the preparation of D-glucosylated enzyme, methods other than by gel permeation were explored. Data are herein presented that demonstrate that dextransucrase immobilized on hydroxylapatite is fully active; this must mean that all of the active sites are available for reaction, assuming that the enzyme is fixed on the solid support in a dispersed manner, so that the molecules may not interact with one another, and thus cannot aggregate. This immobilized enzyme has been used in experiments that demonstrated the formation of a D-glucosylated species by reaction with [^{14}C]sucrose. The radioactive substrate could be rapidly removed from the enzyme by centrifugation or filtration. Labeling

of the enzyme followed saturation kinetics, and an approximation of the concentration required for half maximal rate was similar to K_m values for sucrose[14]. It was possible to release the [^{14}C]saccharides from the immobilized, pulsed enzyme by heating for 10 min at 93°. The sugars were shown to be a mixture of D-glucose and moderate-size oligosaccharides, as had been noted by Robyt *et al.*[1]. Chain-length analysis showed the latter to be ~17 D-glucosyl residues in length, and the fact that these could be released by boiling suggests a relatively labile bond. The nature of this bond requires further investigation.

It may be concluded that, during the exposure to [^{14}C]sucrose, the enzyme becomes labeled with monomeric D-glucosyl groups and oligosaccharides. The oligosaccharides must have developed during the pulse, which helps to explain the high ratio of D-glucose to enzyme, and why the labeling of the enzyme continues over an extended period.

The fact that monomeric D-glucosyl residues are observed on the enzyme is of interest, because, if the model for dextransucrase action proposed by Robyt *et al.*[6] is accurate, it would suggest that any monomeric D-glucosyl units which become bound to the enzyme ought to undergo polymerization. Furthermore, their model would predict that there would be an equal number of D-glucosyl residues and polymer chains on the enzyme. The data presented in Table II indicated that there are substantially fewer oligosaccharide chains than D-glucose residues. The reasons for this are at present unclear.

The results reported herein demonstrate that, when dextransucrase reacts with [^{14}C]sucrose, isotope becomes bound to the enzyme in two forms: monomeric and oligomeric D-glucose. In the accompanying article[7], the characteristics of the monomeric D-glucosyl residues are examined.

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