Carbohydrate Research, 121 (1983) 269–278
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

A D-GLUCOSYLATED FORM OF DEXTRANSUCRASE: DEMONSTRATION OF PARTIAL REACTIONS*

GARY A. LUZIO**, VEENA K. PARNAIK***, AND ROBERT M. MAYER
Department of Chemistry, The Ohio State University, 140 West 18th Avenue, Columbus, Ohio 43210 (U.S.A.)

(Received January 24th, 1983; accepted for publication, April 1st, 1983)

ABSTRACT

A D-glucosylated form of dextransucrase, whose preparation and characteristics have just been reported in *Carbohydr. Res.*, was employed in a series of studies designed to explore the question of whether the bound sugars participate in the reactions catalyzed by the enzyme. When exposed to maltose, a good acceptor-substrate, monomeric D-glucosyl groups were rapidly transferred to the disaccharide, affording a trisaccharide. In the absence of an acceptor, monomeric D-glucose was released from the enzyme by hydrolysis. In a reaction with D-fructose, the charged enzyme catalyzed the formation of sucrose. Finally, in the presence of unlabeled sucrose, monomeric D-glucosyl groups were chased into enzyme-associated oligomers. Evidence is also presented which indicates that the various pathways for the bound D-glucosyl groups are competitive. The significance of these observations is discussed.

INTRODUCTION

The preceding paper[1] described the preparation of a D-glucosylated form of dextransucrase that is produced when the enzyme reacts with [^{14}C]sucrose. The radioactivity is present in a mixture of monomeric D-glucose residues with oligosaccharides that have an average chain-length of 17. Several issues concerning the D-glucosylated enzyme needed to be addressed. Specifically, it is important to determine whether the saccharide units are bonded covalently or noncovalently. Directly related to this question is the matter of whether the saccharides are catalytically active. We now describe a series of experiments that examined the latter question in reference to the monomeric D-glucosyl groups.

Dextransucrase is known to catalyze four types of reaction. The best de-

*Supported by USPHS Grant DE 03731.
**Present address: Hercules Chemical Co., Wilmington, Delaware 19899, U.S.A.
***Present address: Center for Cellular and Molecular Biology, Regional Research Laboratory, Hyderabad-500 009, India.

0008-6215/83/$ 03.00 © 1983 Elsevier Science Publishers B.V.

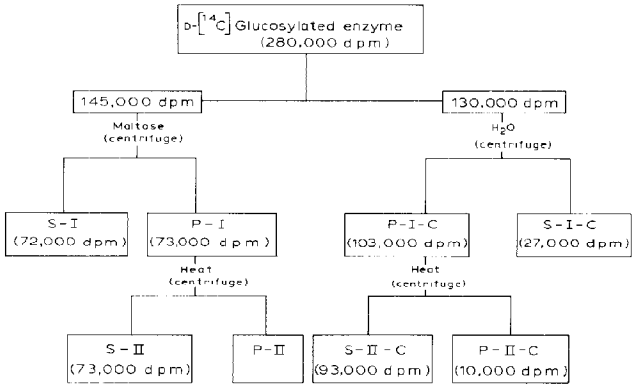
scribed is that in which the D-glucosyl group of sucrose is transferred to nonreducing positions of added, acceptor molecules[2-5]. Multiple additions of single D-glucosyl groups to each acceptor molecule may be viewed as one pathway for polymer formation. The enzyme also appears to catalyze the formation of polymer in the absence of added acceptors[5,6]. It has been suggested[6,7] that this reaction proceeds by addition at the reducing terminus of the growing polymer, which is covalently bonded to the enzyme. In addition, the enzyme has been shown to catalyze the hydrolysis of sucrose[8,9], which is a reaction in which H_2O is the acceptor. The fourth reaction catalyzed by dextransucrase is an isotope-exchange reaction, in which [^{14}C]sucrose can be formed in reactions with unlabeled sucrose and D-[^{14}C]fructose[4,10,11].

The present report focuses on the question of whether the monomeric D-glucosyl groups that are present on the D-glucosylated enzyme can participate in the known reactions. These would constitute a series of partial reactions. The interrelationship between these reactions has also been investigated.

RESULTS

Activity of charged enzyme. — Immobilized enzyme was prepared, and treated with radioactive sucrose in order to generate the "charged" or D-glucosylated form of the enzyme[1]; this was utilized in experiments designed to evaluate the participation of the bound saccharide units in the various reactions catalyzed by the enzyme.

In order to evaluate the ability of the charged enzyme to participate in D-glucosyl-transfer reactions, it was treated with an acceptor according to the protocol described in Scheme 1. The immobilized, charged enzyme was divided into halves at the end of the final wash, and one half was treated with maltose for 1 min, while the other was maintained in buffer for the same length of time. After centrifugation, it was found that more isotope was released into the initial supernatant fluid in the presence of maltose (S-I *vs.* S-IC). Additional isotope was released when pellets P-I and P-IC were heated at 93°. The composition of the supernatant fluids was examined by paper chromatography. Fig. 1, which indicates the products observed in S-I and S-IC, shows that the isotope was distributed in 3 peaks: nonmobile material, which has been shown to consist of oligosaccharides formed during the pulse[1]; material that migrates like a trisaccharide; and glucose. The trisaccharide was present only after exposure to maltose, and was formed by the transfer of enzyme-bound D-glucose to the added disaccharide; this point is illustrated more clearly in Table I, which summarizes the quantitative composition of the supernatant fluids. The major proportion (82%) of the isotope in S-I is comprised of trisaccharide, whereas the corresponding control (S-IC) mainly consists of glucose (92%). Heating of the pellets (P-I and P-IC) released almost all of the residual "counts", and, in both cases, the majority of the isotope-labeled material is immobile on paper chromatograms. In addition, some trisaccharide is released during



Scheme 1. Protocol for maltose chase. [Dextransucrase (12 units) was immobilized on hydroxylapatite as described in Methods. The slurry was pulsed with [^{14}C]sucrose (S.A. = 1.5×10^6 d.p.m./nmol) at a final concentration of 0.05 mM in the presence of mM sodium phosphate buffer, pH 6. At the end of 1 min at room temperature, 5 mL of mM phosphate buffer, pH 6, at 4° was added, and the mixture was centrifuged for 30 s. The pellet was washed twice with 5 mL of the same buffer at 4°. In the final wash, the mixture was divided into two fractions. To one pellet, 0.1M maltose (100 μL) was added, and the mixture was kept for 1 min at room temperature; cold buffer (0.4 mL) was then added, and the mixture centrifuged. The supernatant fluid is designated S-I and the pellet P-I. The second fraction was treated identically, except that H_2O (100 μL) was added, instead of maltose; the supernatant fluid is designated S-IC, and the pellet is P-IC. Pellets P-I and P-IC were resuspended in buffer (500 mL), heated to 93°, and centrifuged. The supernatant fluids derived from this are designated S-II and S-IIC; the pellets are P-II and P-IIC. The numbers in parentheses indicate the radioactivity at each step.]

the heating of P-I; however, this probably represents the presence of some residual maltose that had undergone D-glucosyl transfer subsequent to the removal of S-I, but prior to the heating step. Similar experiments were conducted by the dual-label technique, employing [^{3}H]sucrose and [^{14}C]maltose. The data showed a 1:1 stoichiometry between glucose and maltose in the trisaccharide, and confirmed the initial observations.

The formation of the labeled trisaccharide in the presence of maltose demonstrates that D-glucosyl groups on the charged enzyme participate in the transfer of single groups to acceptors. It must also be pointed out that, in the absence of acceptors, D-glucose is released spontaneously, as seen in S-IC; this is consistent with the concept that water can serve as an acceptor, and this probably constitutes the pathway by which the enzyme catalyzes the hydrolysis of sucrose[8,9].

Another type of acceptor for the enzyme is D-fructose, which reacts to form sucrose[5,10,11]. The reaction may be viewed as an isotope-exchange reaction, as labeled sucrose can be formed in reactions with D-[^{14}C]fructose and unlabeled sucrose. Because this reaction might involve the intermediacy of a D-glucosylated enzyme, an experiment designed to determine whether the charged enzyme could

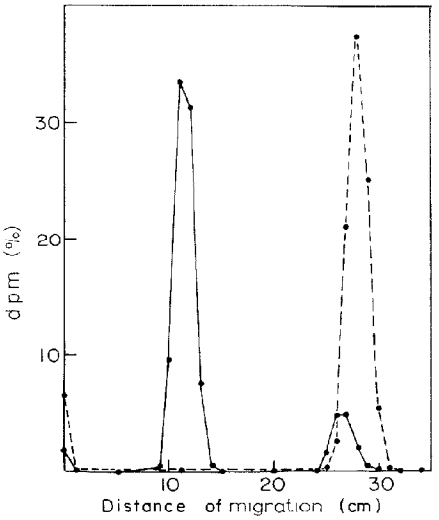


Fig. 1. Paper chromatography of supernatant fluids. [S-I (●——●) 19,400 d.p.m. and S-IC (●---●) 7,780 d.p.m. were spotted on Whatman No. 1 MM paper, and chromatographed in solvent system *II*. The chromatograms were cut into 1-cm strips, and counted by liquid scintillation counting.]

TABLE I

ANALYSIS[a] OF SUPERNATANT FLUIDS

Supernatant fluid	*Origin*		*Trisaccharide*		*Glucose*		*Total d.p.m.*
	d.p.m.	*%*[b]	*d.p.m.*	*%*[b]	*d.p.m.*	*%*[b]	
S-I	2,000	2.7	60,000	82	10,000	13.8	72,000
S-IC	2,000	7.0	>100	0	25,000	92	27,000
S-II	49,000	65.0	12,000	17	12,000	15	73,000
S-IIC	63,000	66.0	>100	0	30,000	32	93,000

[a]The supernatant fluids were chromatographed as described in Fig. 1, and the total radioactivity associated with each of the three components was determined. [b]This is the % of total d.p.m. on the chromatogram.

serve as a source of D-glucosyl groups for the formation of sucrose was performed.

Immobilized enzyme was "charged" in a reaction with [^{14}C]sucrose as described in Scheme 1, and then divided in two. D-Fructose was added to one fraction, and H_2O to the other; after 1 min, the mixtures were heated to 93°, and then analyzed by paper chromatography (see Fig. 2). In the presence of D-fructose (see

TABLE II

AUTOPOLYMERIZATION[a] OF ENZYME-BOUND D-GLUCOSE

	Non-mobile (% total DPM)	D-*Glucose (% total DPM)*
Control	83.8	14.4
Sucrose chase	95.8	3.2
Δ(% d.p.m.)	12.0	11.2

[a]Charged, immobilized enzyme was prepared as described in Scheme 1, and divided in two. To one fraction was added 50mM sucrose (0.5 mL), and after 5 s, the mixture was heated for 10 min at 93°, cooled, and centrifuged. An aliquot (50 μL) of the supernatant fluid was analyzed by paper chromatography in solvent system *I*, and the chromatogram was counted. Water (0.5 mL) was added to the second fraction, which was then treated identically.

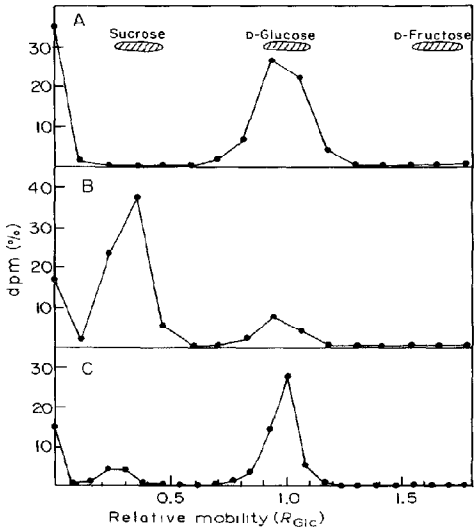


Fig. 2. Formation of sucrose by the "charged" enzyme. [The "charged" enzyme was prepared as described in Scheme 1, utilizing 3.4 units of immobilized dextransucrase, and the sample was divided into two approximately equal fractions. (A) To one fraction was added 0.1M D-fructose, and the mixture was kept for 1 min at room temperature. The sample was heated for 2 min at 93°, cooled, H_2O (0.4 mL) added, and the mixture centrifuged. An aliquot (50 μL) of the supernatant liquor was analyzed by paper chromatography in solvent system *I*. (B) The second fraction was treated identically, except that 100 μL of mM phosphate buffer, pH 6, was used instead of D-fructose. (C) An aliquot (50 μL) of the supernatant liquor in (A) was treated with invertase (0.07 unit) for 15 min, and the products were analyzed by paper chromatography in solvent system *I*.]

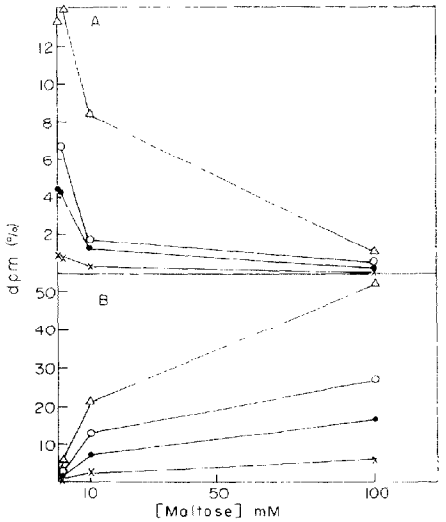


Fig. 3. Competition between D-glucosyl transfer and autopolymerization. [Dextransucrase (0.25 unit) was incubated with 6.5 μl. of [^{14}C]sucrose (1.32 × 10^6 d.p.m.) and with either 0, 0.065, 0.65, or 6.5 μmol of maltose and sodium phosphate buffer, pH 6, in a total volume of 65 μl. The reactions were stopped at 1 (×), 5 (●), 10 (○), and 30 (△) min by heating for 2 min at 100°. Aliquots were analyzed by paper chromatography in solvent system *II*, and the chromatograms counted. (A) The percentage of the total d.p.m. at the origin, or (B), in the acceptor product regions, was calculated, and plotted as a function of the concentration of maltose.

Fig. 2A), [^{14}C]sucrose was formed, whereas none was detected in its absence (see Fig. 2B). When the reaction products formed in the reaction with D-fructose were exposed to invertase, the [^{14}C]sucrose was converted into D-[^{14}C]glucose (see Fig. 2C) and D-fructose. In the control for this reaction, in which heat-denatured invertase was utilized, no cleavage of [^{14}C]sucrose was observed. It may be concluded that the charged enzyme can also serve as a source of D-glucosyl groups for the formation of [^{14}C]sucrose.

Dextransucrase also catalyzes the autopolymerization of the D-glucosyl groups of sucrose molecules in the absence of added acceptors. This reaction may constitute a pathway for the *de novo* synthesis of dextran. An experiment was conducted in order to ascertain whether D-glucosyl groups on the charged enzyme could be chased into polymer. Table II describes the results observed when D-[^{14}C]glucose-charged enzyme was chased with unlabeled sucrose for 5 s. It may be seen that the proportion of monomeric D-[^{14}C]glucosyl groups on the enzyme de-

creased with an equivalent increase in the level of isotope in the nonmobile fraction. These data indicate that the D-glucosyl groups can be incorporated into the polymeric forms.

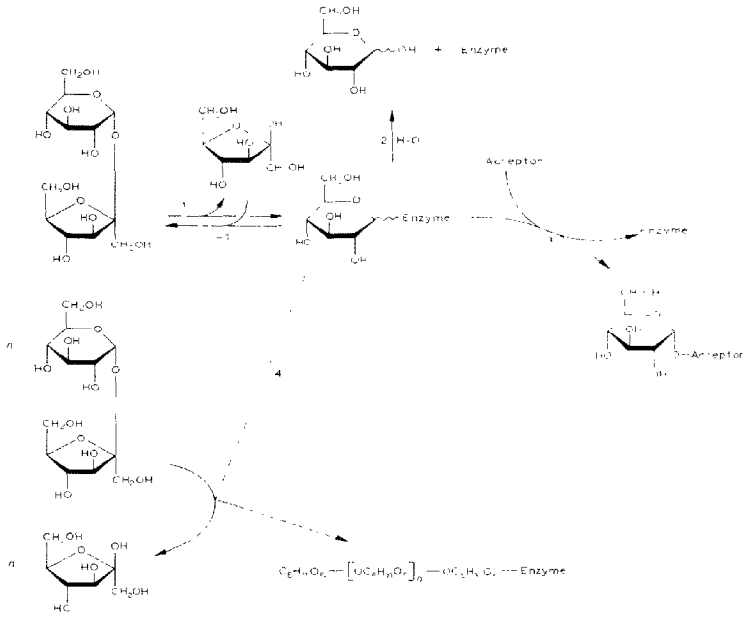
Competition between D-*glucosyl transfer and autopolymerization.* — The data presented in Fig. 1 indicate that, when an acceptor is present, the monomeric D-glucosyl groups on the charged enzyme are partitioned between the transfer and the autopolymerization reactions; this conclusion could be drawn, as the products of both pathways were observed. However, the amount of polymer formed when an acceptor was present was less than in its absence. This observation raised the possibility that the two reaction-pathways compete for the same D-glucosyl groups. To examine this question in more detail, an experiment was conducted in which the enzyme reacted with various concentrations of maltose, and the amounts of the transfer and polymerization products were measured. The results (see Fig. 3) showed that, as the acceptor concentration was increased, more product appeared in transfer products and less in the polymer. This type of reciprocal behavior indicates that the two pathways are competitive.

DISCUSSION

We had already demonstrated[1] that dextransucrase could be labeled with D-[^{14}C]glucosyl groups during reaction with [^{14}C]sucrose. The radioactive sugars were present on the enzyme as monomeric D-glucosyl groups, and as oligosaccharides having an average chain length of 17. We have now examined the question of whether these saccharide units can participate in the reactions catalyzed by the enzyme.

The results of these studies are summarized in Scheme 2, which is a reaction pathway proposed for dextransucrase action. Central to this scheme is the involvement of an intermediate that contains monomeric D-glucosyl groups on the enzyme; this is formed in the D-glucosylation reaction (Reaction 1), and the reaction can be reversed in the presence of D-fructose. The latter step illustrates the mechanism by which dextransucrase catalyzes the isotope-exchange reaction[5,10,11]. In reaction 2, the hydrolysis of the D-glucosyl groups from the charged enzyme, is demonstrated. The sequence of reactions 1 and 2 constitutes the pathway by which dextransucrase catalyzes the hydrolysis of sucrose[8,9]. The transfer of single D-glucosyl groups to acceptors is described by a combination of reactions 1 and 3. We have been able to show that the D-glucosyl groups on the charged enzyme can participate in reaction 3. In addition, the rates of transfer from the charged species and from sucrose to an acceptor are approximately equivalent. Finally, the pathway for autopolymerization is described by reactions 1 and 4. The fact that monomeric D-glucosyl groups can be chased into polymer illustrates that this reaction can occur. It is presumed that this proceeds by insertion, as suggested by Robyt *et al.*[6].

An important element in Scheme 2 is the hypothesis that the same



Scheme 2 Proposed reaction-pathways for dextransucrase.

monomeric units have the potential to enter each of the catalytic pathways. This concept is based on the results of a series of experiments that demonstrated competition between the pathways. We had previously shown that both D-glucosyl transfer and autopolymerization compete with the hydrolysis of sucrose[9]. We have now demonstrated that D-glucosyl transfer and autopolymerization compete with each other. Earlier work[11a] in our laboratory had indicated that D-glucosyl transfer and the isotope-exchange reaction also compete. These observations suggest that the pathways compete for a form of the enzyme that is common to all of the pathways, and we now propose that this is the D-glucosylated form.

The nature of the bonding involved with the monomeric D-glucosyl units has not been determined, but the data presented here establish that they are activated toward transfer; this suggests that the bond is covalent, and is a high-energy bond. The observation that D-glucosyl groups are hydrolyzed at room temperature, and at 93°, is also consistent with a high-energy bond. The characteristics of the bond

between the enzyme and the oligosaccharide units are also unclear; however, it is possible that these will undergo hydrolysis and transfer *en bloc*, as suggested by Robyt and Walseth[12]. The fact that these chains are released at 93° may illustrate a type of bonding that is analogous to that of the D-glucosyl groups.

A major question raised by these studies is: why is it possible to isolate a mono-D-glucosylated form of the enzyme at all? It would be expected that such groups would spontaneously enter the autopolymerization pathway. The fact that this occurs rapidly when sucrose is present (see Table II) may indicate that the pathway requires the presence of sucrose.

An important element in these studies is the rapidity of the pulse, and the isolation and utilization of the charged form of the enzyme. The hydrolysis of monomeric D-glucosyl groups occurs at a sufficiently high rate to preclude the utilization of inordinately long reaction-times or work-up times, such as have been reported by other investigators[6,12].

The results described in this and the accompanying report[1] describe the preparation of a charged derivative of dextransucrase, and its utilization in studies on the catalytic pathways.

EXPERIMENTAL

Materials. — Dextransucrase was purified by a modification of the procedure of Huang *et al.*[13]. The enzyme was free from extraneous proteins; however, multiple forms of the enzyme were present[14]. The enzyme preparations had specific activities ranging from 80 to 100 units/mg. [^{14}C]Sucrose and [^{3}H]sucrose were purchased from New England Nuclear Corp. (Boston, MA), as was [^{14}C]maltose, which was purified to radiochemical purity by paper chromatography in solvent system *II*. Hydroxylapatite was purchased from Sigma Chemical Co. (St. Louis, MO). All other enzymes and reagents were purchased from commercial sources and were of reagent quality.

Methods. — Enzyme activity was measured as previously described[14]. Immobilized enzyme was prepared by the procedure described by Parnaik *et al.*[1]. The D-glucosylated form of the enzyme was prepared by incubating the immobilized enzyme with 0.05mM [^{14}C]sucrose, or [^{3}H]sucrose (S.A. = 1.5×10^6 d.p.m./nmol). After 1 min at room temperature, the mixture was diluted with 5 mL of mM sodium phosphate buffer, pH 6.0, at 4°, and centrifuged for 30 s. The pellet was washed with several 5-mL volumes of the same buffer, and resuspended in buffer (0.5 mL), and an aliquot was removed for counting.

Paper chromatography was conducted in solvent system *I*, namely, 9:1:1 (v/v) butanone–acetic acid–H_2O saturated with boric acid, or in solvent system *II*, 6:1:3 (v/v) 1-propanol–ethyl acetate–H_2O. Radioactivity was measured as previously described[1].

REFERENCES

1 V. K. PARNAIK, G. A. LUZIO, D. A. GRAHAME, S. L. DIPSON, AND R. M. MAYER, *Carbohydr. Res.*, 121 (1983) 257–268.
2 E. J. HEHRE, *J. Polym. Sci., Part C*, 23 (1968) 239–244.
3 G. J. WALKER, *J. Dent. Res.*, 51 (1972) 409–414.
4 G. J. WALKER, *Carbohydr. Res.*, 30 (1973) 1–8.
5 R. M. MAYER, M. M. MATTHEWS, C. L. FUTERMAN, V. K. PARNAIK, AND S. M. JUNG, *Arch. Biochem. Biophys.*, 208 (1981) 278–287.
6 J. F. ROBYT, B. K. KIMBLE, AND T. F. WALSETH, *Arch. Biochem. Biophys.*, 165 (1974) 634–640.
7 K. H. THIELE AND G. SCHENK, *Adv. Enzymol.*, 30 (1968) 179.
8 A. GOODMAN, R. M. WEIS, AND K. G. STERN, *J. Biol. Chem.*, 217 (1955) 977–985.
9 G. A. LUZIO AND R. M. MAYER, *Carbohydr. Res.*, 111 (1983) 311–318.
10 E. J. HEHRE AND H. SUZUKI, *Arch. Biochem. Biophys.*, 113 (1966) 675–683.
11 E. J. BOURNE, J. PETERS, AND H. WEIGEL, *J. Chem. Soc.*, (1964) 4680–4687.
11a S. HUANG, unpublished results.
12 J. F. ROBYT AND T. F. WALSETH, *Carbohydr. Res.*, 61 (1978) 433–445.
13 S. HUANG, H. C. LEE, AND R. M. MAYER, *Carbohydr. Res.*, 74 (1979) 287–300.
14 G. A. LUZIO, D. A. GRAHAME, AND R. M. MAYER, *Arch. Biochem. Biophys.*, 216 (1982) 751–757.