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Dextransucrase and the mechanism for dextran biosynthesis

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ABSTRACT

Remaud-Simeon and co-workers [Moulis, C.; Joucla, G.; Harrison, D.; Fabre, E.; Potocki-Veronese, G.; Monsan, P.; Remaud-Simeon, M. *J. Biol. Chem.*, **2006**, *281*, 31254–31267] have recently proposed that a truncated *Escherichia coli* recombinant B-512F dextransucrase uses sucrose and the hydrolysis product of sucrose, D-glucose, as initiator primers for the nonreducing-end synthesis of dextran. Using ^{14}C-labeled D-glucose in a dextransucrase–sucrose digest, it was found that <0.02% of the D-glucose appears in a dextran of M_n 84,420, showing that D-glucose is not an initiator primer, and when the dextran was treated with 0.01 M HCl at 80 °C for 90 min and a separate sample with invertase at 50 °C for 24 h, no D-fructose was formed, indicating that sucrose is not present at the reducing-end of dextran, showing that sucrose also was not an initiator primer. It is further shown that both D-glucose and dextran are covalently attached to B-512FMC dextransucrase at the active site during polymerization. A pulse reaction with [^{14}C]-sucrose and a chase reaction with nonlabeled sucrose, followed by dextran isolation, reduction, and acid hydrolysis, gave ^{14}C-glucitol in the pulsed dextran, which was significantly decreased in the chased dextran, showing that the D-glucose moieties of sucrose are added to the reducing-ends of the covalently linked growing dextran chains. The molecular size of dextran is shown to be inversely proportional to the concentration of the enzyme, indicating a highly processive mechanism in which D-glucose is rapidly added to the reducing-ends of the growing chains, which are extruded from the active site of dextransucrase. It is also shown how the three conserved amino acids (Asp551, Glu589, and Asp 622) at the active sites of glucansucrases participate in the polymerization of dextran and related glucans from a single active site by the addition of the D-glucose moiety of sucrose to the reducing-ends of the covalently linked glucan chains in a two catalytic-site, insertion mechanism.

1. Introduction

In 1941 Hehre[1] reported the first cell-free synthesis of dextran using sucrose as the substrate. Shortly thereafter, the enzyme dextransucrase [EC 2.4.1.5] was named as the enzyme responsible for the synthesis. It was found that both *Leuconostoc mesenteroides* strains and *Streptococcus* sp. elaborated dextransucrases. Dextransucrase actually is a generic name for a family of enzymes that synthesize dextrans with different structures from sucrose. Dextrans are glucans with contiguous α-(1→6) glucosidic linkages in the main chains that have various arrangements (random, continuous, and alternating single glucose residues and long dextran branched chains with three different kinds of branch linkages [α-(1→2), α-(1→3), or α-(1→4)], depending on the dextransucrase that is produced by the specific strain of *L. mesenteroides* or *Strep.* sp. In 1954, Jeanes et al.[2] reported the synthesis of many different kinds of dextrans by 96 strains of *L. mesenteroides* and *Streptococcus* sp. Both types of bacteria are Gram-positive cocci that are closely related. *L. mesenteroides* strains are facultative anaerobes, while *Streptococci* are strict anaerobes. Another notable difference between them is that *L. mesenteroides* strains require sucrose in their culture medium to induce the formation of the glucansucrases, whereas *Streptococcus* sp. are constitutive for glucansucrases and do not require sucrose in their medium for their elaboration. In 1994, Kim and Robyt,[3] using ethyl methanesulfonate as a mutagen, reported the mutation and selection of several *L. mesenteroides* strains (B-512FMC, B-742CA, B-742CB, B-1142C, B-1299C, B-1355CA, and B-1355CB) that were constitutive for their glucansucrases and did not require sucrose in their culture medium for

Abbreviations: DS, dextransucrase; LMW, low molecular weight; HMW, high molecular weight; M_n, number average molecular weight; DP, degree of polymerization; HA, hydroxyapatite; HA–DS, hydroxyapatite immobilized dextransucrase; CP, conversion period, which is the theoretical time necessary to convert the substrate into product for the amount of enzyme present; B-512FMC, dextransucrase constituent mutant of *Leuconostoc mesenteroides* B-512F; APTS, 8-amino-1,3,5-pyrenetrisulfonic acid, derivatizing fluorescent reagent; SDS, sodium dodecylsulfate; TFA, trifluoroacetic acid.

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induction and elaboration of their glucansucrases. The glucansucrases that were secreted into the culture medium were thus devoid of glucan and other products produced by glucansucrase. The mutants also were selected to elaborate only one of the two glucansucrases for those wild-type organisms that elaborated two kinds of glucansucrases. The mutant glucansucrases synthesized glucans that had the same structure and characteristics as the corresponding glucansucrases that were produced by the wild-type organisms. All of the mutants also produced 3–22 times the activity when grown on D-glucose than the amount produced by the wild-type parent strains when grown on sucrose.

Besides the synthesis of dextrans, dextransucrases also catalyze secondary transglycosylation reactions in which the D-glucose moiety of sucrose is transferred to mono- and oligosaccharides present or added to the digest to give oligosaccharide products.[4–7] This is called an 'acceptor reaction' and occurs at the expense of dextran synthesis.[7] The acceptor reactions also involve (a) the transfer of D-glucose to an acceptor monosaccharide or oligosaccharide,[6,7] (b) the transfer of D-glucose to a dextran chain to give D-glucosyl branch linkages, and the transfer of the dextranyl chain to a dextran chain to give dextranyl branched dextran chains,[8] (c) a very minor acceptor reaction in which the D-glucose moiety of sucrose is transferred to water to give the hydrolysis of sucrose,[9] (d) and the transfer of the dextranyl chain to water and/or to an acceptor saccharide (D-glucose, D-D-fructose, sucrose, or maltose) to release the dextran from the active site and terminate polymerization.[6,9]

In 1974 Robyt et al.,[10] using pulse and chase experiments with [^{14}C]-sucrose, showed that Bio-Gel P2-immobilized dextransucrase was transferring D-glucose from sucrose to the reducing-ends of the growing dextran chains. The enzyme formed covalent intermediates with the C1–OH of D-glucose and with the C1–OH at the reducing-end of the growing dextran chain. A two-site insertion mechanism was proposed for the synthesis of dextran in which D-glucose is inserted between the enzyme and the reducing-end of the growing dextran chain by means of the transfer of the dextran chain to the covalent D-glucosyl unit. In 1983, Robyt and Martin,[11] also using pulse and chase techniques with *Streptococcus mutans* 6715 dextransucrase (GTF-S) and mutansucrase (GTF-I), showed that the two *S. mutans* glucans, dextran and mutan, were also synthesized by the two enzymes from the reducing-end of the growing glucan chains. In 1984, Ditson and Mayer[12] also showed that the synthesis of dextran by *S. sangius* dextransucrase was from the reducing-end by the two-site insertion mechanism and not by a primer, nonreducing-end mechanism.

For over 20 years, 1956–1976, various investigators searched for an enzyme that branched dextran. A branching enzyme was never found. In 1976, Robyt and Taniguchi[8] showed that D-glucose and a dextran chain, covalently linked to B-512F dextransucrase, were released from the active site by exogenous dextran chains to form α-(1→3) branch linkages. They showed that the biosynthesis of the branch linkages did not require a separate branching enzyme and took place by an exogenous acceptor dextran chain, displacing the covalent glucosyl–dextransucrase complex or displacing the covalent dextranyl–dextransucrase complex to give α-(1→3) branched single glucose units and α-(1→3) branched dextran chains, respectively.

In 1978, Robyt and Walseth[6] showed that the mechanism of the acceptor reactions was the attack of the acceptor on the D-glucosyl- and dextranyl-covalent complexes, releasing them from the active sites of B-512F dextransucrase. In 1983, Robyt and Eklund[7] reported the relative quantitative effects of 17 saccharide acceptors. Maltose was the best acceptor, followed by isomaltose, D-glucose, and D-fructose in that order, and that the increase in the concentration of maltose gave an exponentially decreasing amount of dextran, indicating the inhibition of dextran synthesis.

Recently in 2006, there appeared a paper in the *Journal of Biological Chemistry* by Remaud-Simeon and co-workers,[13] using N- and C-terminal truncated *L. mesenteroides* B-512F dextransucrase that was cloned in *E. coli*. They state that this enzyme synthesizes B-512F dextran by a nonprocessive or semiprocessive reaction in which D-glucose and sucrose act as initiator primers and the D-glucose moiety of sucrose is added to the C-6–OH of D-glucose and to the C-6–OH of the D-glucose moiety of sucrose to give dextran polymerization from a single active site by the addition to the nonreducing-ends of isomaltodextrins and not by the two-site insertion mechanism, as had previously been established by several studies.

In the present study, we show that D-glucose produced by the hydrolysis of sucrose, and sucrose itself are not initiator primers, and that D-glucose is not added to the nonreducing-ends of isomaltodextrin primers for the synthesis of HMW dextran, as proposed by Remaud-Simeon and co-workers.[13] Using pulse and chase experiments, we reaffirm that D-glucose from sucrose is added to the reducing-end of a growing dextran chain. We also reaffirm that D-glucosyl- and dextranyl-covalent enzyme complexes are formed during the polymerization reaction and that the enzyme catalyzed reaction is highly processive and not nonprocessive or semi-processive, as was proposed by Remaud-Simeon and co-workers.[13] Further, we show how only one set of the three amino acids (Asp-551, Glu-589, and Asp-622), known to be conserved at the active sites of the so-called GH-family 70 glucansucrases, can participate in the two catalytic-site, insertion mechanism for the polymerization of glucans by glucansucrases, also contrary to the claim by Remaud-Simeon and co-workers[13] that there would have to be two sets of the conserved amino acids to have participation in a two-site mechanism.

2. Experimental

2.1. Materials

2.1.1. Chemicals

(a) [^{14}C]-D-glucose (1 mCi/mL) was purchased from New England Nuclear in 1975 and kept frozen in the laboratory, until needed; (b) [^{14}C]-UL-sucrose (0.1 mCi/mL) was obtained from Sigma Chemical Co. (St. Louis, MO, USA); (c) isomaltodextrin standards were obtained by acceptor reaction of 75 U of *L. mesenteroides* B-512FMC in 10 mL of 200 mM sucrose and 200 mM D-glucose incubated for 24 h at 23 °C. Excess D-glucose was removed by immobilized yeast;[14] 5 mL of ethanol was added to precipitate dextran, the mixture was centrifuged, and the supernatant was concentrated to 2 mL; the individual isomaltodextrins were purified on BioGel P-2 and P-4 (fine) columns (2.5 × 120 cm), using water as an eluant; (d) ^{14}C was counted in Bray's dioxane cocktail for aqueous samples, and toluene cocktail was used to count the radioactivity of carbohydrates adsorbed onto Whatman 3MM paper.[15]

2.1.2. Enzymes

(a) *L. mesenteroides* B-512FMC dextransucrase (hereinafter B-512FMC dextransucrase) was obtained from *L. mesenteroides* B-512FMC constitutive mutant.[3,16] The culture supernatant was concentrated and dialyzed against 20 mM pyridinium acetate buffer (pH 5.2) on an ultrafiltration, hollow-fiber cartridge (10 cm × 1 m, H5P100-43, Amicon, Inc., Beverly, MA).[17] The B-512FMC dextransucrase activity was 520 IU/mg, using the ^{14}C-sucrose assay, described below, where 1.0 IU = 1.0 μmol of D-glucose incorporated into dextran per min; (b) invertase from baker's yeast was obtained from Sigma Chemical Co.