Carbohydrate Research, 126 (1984) 170–175
Elsevier Science Publ shers B.V., Amsterdam – Printed in The Netherlands

Note

Dextransucrase: The direction of chain growth during autopolymerization*

SUSAN L. DITSON AND ROBERT M. MAYER
Department of Chemistry, The Ohio State University, Columbus, OH 43210 (U S.A.)
(Received September 10th, 1983; accepted for publication, October 1st, 1983)

The formation of dextran from sucrose can proceed by two reaction pathways, both of which are catalyzed by dextransucrase (EC 2.4.1.5). One pathway involves chain elongation *via* sequential transfers of D-glucosyl groups to nonreducing positions of acceptor saccharides[1–4]. The second pathway, which occurs in the absence of added acceptors, is considered to be an autopolymerization reaction, and may account for the *de novo* synthesis of dextran[4–7]. Previous studies[6,7] indicated that the dextranyl chains formed may be bound to the enzyme. The early experiments by Robyt *et al.*[6] provided evidence that supports the proposal that chain growth occurs at the reducing terminus. In their work, a crude enzyme preparation, immobilized by chemical cross-linking to Bio-Gel P-2, was treated with a low concentration of [^{14}C]sucrose for 10 h, during which time, the isotope became bound. Analyses showed that the bound isotope was a mixture of D-glucose and dextranyl chains. When the labeled enzyme was subsequently reacted with a high concentration of unlabeled sucrose, radioactivity at the reducing terminus diminished, and the conclusion was drawn that chain growth occurs at the reducing end.

Several criticisms have been made of these studies[8]. Firstly, the crudeness of the enzyme preparation, and the fact that the cells had been grown in the presence of sucrose, call into question the availability of endogenous, acceptor chains. Secondly, the cross-linking process yielded an enzyme that retained only 1% of the original activity. Finally, the extended reaction-time with [^{14}C]sucrose cannot be considered to be representative of a catalytic process.

Recently, we have obtained[9] highly purified enzyme preparation from *S. sanguis* ATCC-10558, and have developed a procedure for immobilization of the enzyme, such that a minimum of 80% of the activity is expressed in the immobilized state[8]. With these developments, we considered it important to reexamine the issues raised by the work of Robyt *et al.*[6], and now report the results of these studies.

*Supported, in part, by USPHS Grant No. DE03731.

0008-6215/84/$ 03.00

RESULTS AND DISCUSSION

In order to examine the matter of the direction of chain growth during the autopolymerization of dextran, enzyme preparations that showed >90% homogeneity were employed. The enzyme was immobilized on hydroxylapatite by a published procedure[8]. This procedure results in a minimum of 80% of the original activity being expressed in the immobilized state. The immobilized enzyme was treated with [^{14}C]sucrose, and then the unreacted substrate and the soluble product (D-fructose) were rapidly removed by a series of three washes and centrifugations. The total elapsed time from the addition of tracer to the completion of the washes was <5 min. Under these conditions, the concentration of radioactive sucrose in the immobilized enzyme was <1 nmol. The radioactive components, which remained with the enzyme, were released as described in the Experimental section. As previously observed[7], they were a mixture of D-glucose and D-gluco-oligosaccharides.

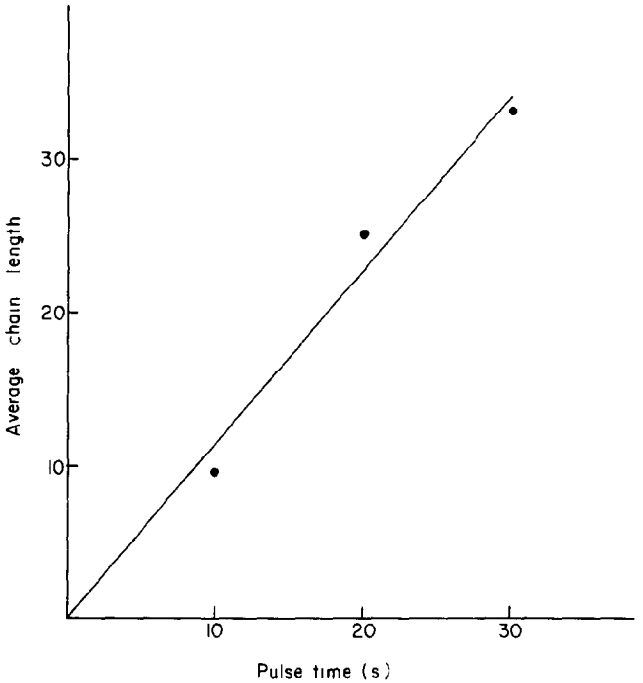

Fig. 1. The average chain-length of oligosaccharides formed during pulse. [Immobilized enzyme (6 units) reacted with 0.3 mL of [^{14}C]sucrose (2.4×10^7 d.p.m.), as described in the Experimental section. Aliquots (100 μL) were withdrawn at 10, 20, and 30 s, and added to chilled water (5 mL). After three rapid washes, the bound products were released from the enzyme by the procedure described in the Experimental section. The oligosaccharide fractions isolated by chromatography on Bio-Gel P-30 were reduced with 0.54M $NaBH_4$ for 14 h at 50°. The samples were made neutral, and hydrolyzed in M HCl for 5 h at 95°. The hydrolyzates were analyzed by paper chromatography, and the average chain-length determined from the ratio of (glucose + glucitol)/glucitol.]

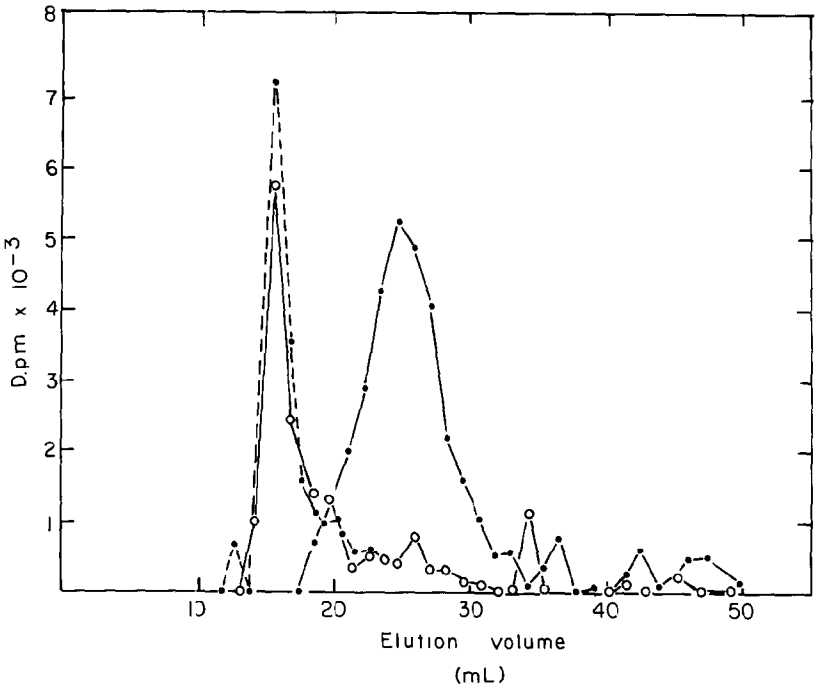

Fig. 2. Gel chromatography of enzyme-bound products following the chase with cold sucrose. [Immobilized enzyme (8 units) was "charged" for 10 s, as described in the Experimental section. The washed pellet was resuspended in 50mM unlabeled sucrose (0.4 mL) at 4°. Aliquots (100 μL) were withdrawn at 0 (●—●), 5 (○-○), 15 (●---●), 25, 45, and 60 s, and the bound products released as described in the Experimental section, and then chromatographed on Bio-Gel P-30.]

The formation of the oligosaccharides was monitored as a function of the length of time of reaction with [^{14}C]sucrose. Following release of radioactive products, the oligosaccharides were isolated by gel chromatography on Bio-Gel P-30, and subjected to chain-length analysis by reduction with $NaBH_4$ followed by acid hydrolysis. The resultant D-glucose and D-glucitol were separated by paper chromatography, and the average chain-length was calculated[8]. These data are plotted in Fig. 1, which shows an increase in chain length as a function of time.

To examine the direction of chain growth, immobilized enzyme was "charged" for 10 s, rapidly washed, and then treated with a high concentration of unlabeled sucrose. Samples were withdrawn at several elapsed times, and, following release of the labeled products, the samples were chromatographed on Bio-Gel P-30, as shown in Fig. 2. It may be seen that the relative, molecular weight increased as a function of time of reaction with the unlabeled sucrose.

If chain growth during the cold chase occurred at reducing termini, the newly incorporated, or unlabeled D-glucosyl residues, would be located at those positions. Thus, it would be expected that any isotope at the reducing ends following the "charging" reaction would be displaced. In order to evaluate this matter, the

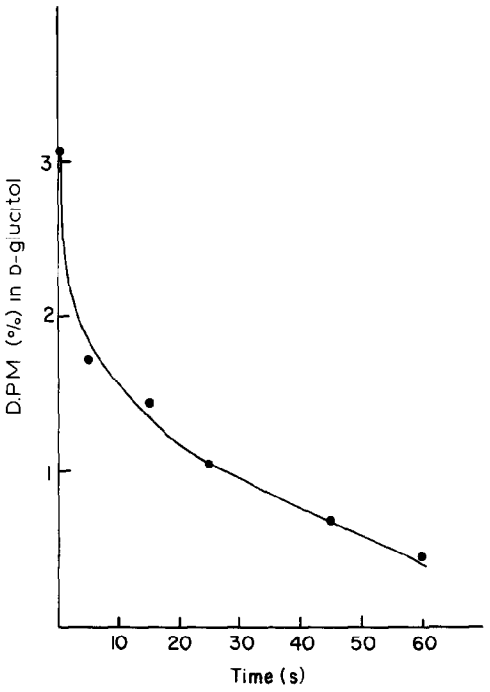

Fig. 3. Reducing-end analysis of cold chase products. [The oligosaccharides isolated as described in Fig. 2 were reduced, and the products hydrolyzed as described in Fig. 1. Following paper chromatography, the radioactivity in the D-glucitol was measured, and plotted as a function of time.]

oligosaccharides obtained as described in Fig. 2 were reduced with $NaBH_4$, to convert the reducing ends into D-glucitol residues. The radioactivity in the D-glucitol was measured following acid hydrolysis and paper chromatography. These data, plotted in Fig. 3, show that there was a rapid decline in the level of isotope in the D-glucitol during the chase with cold sucrose.

These data indicate that chain growth *does* occur at the reducing terminus of a growing chain during the autopolymerization reaction. This supports the proposals of Ebert and Schenk[5] and Robyt *et al.*[6], who suggested that the mechanism of chain elongation in this reaction involves insertion of new monomers between the reducing end and the enzyme. It has been established that the enzyme catalyzes two types of chain elongation reactions that compete with each other for a common intermediate[7], one being D-glucosyl transfer to acceptors, and the other, the autopolymerization reaction. It thus appears that the two principal chain-elongation reactions differ in the direction of chain growth, and as regards their dependence on added acceptors.

EXPERIMENTAL

General. — Dextransucrase was obtained, and purified, as described previously[8]. Bio-Gel P-30 was purchased from Bio-Rad Laboratories (Richmond, CA), and hydroxylapatite (25% of solid in suspension), from Sigma Chemical Co. (St. Louis, MO). New England Nuclear (Boston, MA) was the source of [U-^{14}C]sucrose. All other chemicals were purchased from common suppliers, and were of reagent grade.

Immobilization of enzyme. — Dextransucrase was immobilized on hydroxylapatite (HA) by a modification of the procedure described previously[8]. To each 0.1 mL of a 25% suspension of HA was added enzyme (2 units) and the mixture was kept for 30 min at 4°. Following centrifugation, and removal of the supernatant fluid, the enzyme was ready for use. In all cases, the immobilized enzyme retained a minimum of 85% of its original activity.

Charging of immobilized enzyme. — The immobilized enzyme was "charged" by the addition of 0.1 mL of 0.05mM [^{14}C]sucrose (8×10^7 d.p.m./mL) in mM sodium phosphate buffer, pH 6.0, containing, for every 2 units of immobilized enzyme, 50 μg of Triton X-100/mL. The mixture was immediately stirred, and kept for 10 s at 4°, unless otherwise noted. The concentration of sucrose was lowered by addition of water (5 mL) prechilled to 4°, and the samples were centrifuged for 20 s. Following removal of the supernatant fluid, the pellets were rapidly washed and centrifuged (three times). The total, elapsed time to completion of the washes was 4.5–5 min, and the amount of [^{14}C]sucrose remaining in the pellets was <1 nmol.

Release of bound products. — Products bound to the immobilized enzyme were released by addition of a 0.5% solution of sodium dodecyl sulfate (0.5 mL per 0.1 mL of immobilized enzyme). The samples were incubated for 10 min at room temperature, and centrifuged. To remove the SDS from the supernatant fluids, 0.1 mL of saturated KCl was added, and after 5 min at 4°, the samples were centrifuged to remove the precipitate of potassium dodecyl sulfate. Over 85% of the bound isotope was recovered, except in instances in which very prolonged reaction-times had been employed.

Analytical procedures. — Paper chromatography was conducted on Whatman No. 1 MM paper in 9:1:1 (v/v) butanone–acetic acid–water saturated with boric acid. Gel filtration was performed on columns (1×45 cm) of Bio-Gel P-30, using water as the eluant. Measurements of radioactivity were made in a liquid scintillation counter. Aqueous samples were counted in Insta-Gel (Packard Instrument Co., Downers Grove, IL); paper chromatograms were cut into strips (1×2.4 cm), and counted as previously described[4]. Dextransucrase activity was measured by the procedure described by Luzio *et al.*[10].

REFERENCES

1 G. J. WALKER, *J. Dent. Res.*, 51 (1972) 409–414.
2 G. J. WALKER, *Carbohydr. Res.*, 30 (1973) 1–8.

3 E. J. HEHRE, *J. Polym. Sci.*, 23 (1968) 239–244.
4 R. M. MAYER, M. M. MATTHEWS, C. L. FUTERMAN, V. K. PARNAIK, AND S. M. JUNG, *Arch. Biochem. Biophys.*, 208 (1981) 278–287.
5 K. H. EBERT AND G. SCHENK, *Adv. Enzymol.*, 30 (1968) 179–221.
6 J. F. ROBYT, B. K. KIMBLE, AND T. F. WALSETH, *Arch. Biochem. Biophys.*, 165 (1974) 634–640.
7 G. A. LUZIO, V. K. PARNAIK, AND R. M. MAYER, *Carbohydr. Res.*, 121 (1983) 269–278.
8 V. K. PARNAIK, G. A. LUZIO, D. A. GRAHAME, S. L. DITSON, AND R. M. MAYER, *Carbohydr. Res.*, 121 (1983) 257–268.
9 D. A. GRAHAME AND R. M. MAYER, unpublished results.
10 G. A. LUZIO, D. A. GRAHAME, AND R. M. MAYER, *Arch. Biochem. Biophys.*, 216 (1982) 751–757.