BIOSYNTHESIS OF OLIGOSACCHARIDE COMPONENTS OF CRYPTOCOCCUS LAURENTII CELL WALL

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1. Introduction

Recent investigations in this laboratory have shown that the cell wall of *Cryptococcus laurentii* var flavescens NRRL Y-1401 contains at least two heteropolysaccharide components: a neutral heteropolysaccharide composed of mannose*, galactose, xylose, and L-arabinose and an acidic heteropolysaccharide containing mannose, glucuronic acid and xylose. The latter is similar in composition to the acidic extracellular polysaccharide that can be isolated from the culture medium.

A particulate enzyme preparation from *C. laurentii* has been shown to incorporate ^{14}C-mannose from GDP-^{14}C-mannose into an endogenous acceptor that is similar in composition to the neutral cell wall polysaccharide [1]. Degradation of the product labeled with ^{14}C-mannose allows the isolation of two radioactive mannosyl dissacharides, α-1,2 mannobiose and α-1,3 mannobiose [2].

During further investigation of the biosynthesis of the cell wall of *C. laurentii* it was discovered that various mono- and oligosaccharides could act as acceptors for the transfer of mannose and xylose from GDP-mannose and UDP-xylose respectively. These studies are described in this communication.

2. Methods and materials

The enzyme was prepared from *C. laurentii* essentially as described by Cohen and Feingold [3; fraction P2] except that 0.1 M Tris-HCl buffer, pH 7.3, was substituted for phosphate buffer. The reaction catalyzing the synthesis of oligosaccharides from the appropriate acceptor was assayed as follows: reaction mixtures containing 0.05 μC of either GDP-^{14}C-mannose (151 mC/mmole), or UDP-^{14}C-xylose (172 mC/mmole), $MnCl_2$ (0.5 μmoles), enzyme (0.25 mg protein), and acceptor (0.5–2.0 μmoles) in a total volume of 40 μl of 0.1 M sodium acetate buffer, pH 6.3, were incubated at 25° for 2 hr. The reaction mixtures were then applied to Whatman No. 1 filter paper and subjected to chromatography in either ethyl acetate-acetic acid-water, 3 : 1 : 1 (solvent A) or n-propanolethyl acetate-water, 7:1:2 (solvent B) for 48–96 hr. The reaction products were located on chromatograms with the use of a paper strip scanner or by autoradiography.

Authentic α-1,2-mannobiose and α-1,2-mannotriose (containing mainly Man-α-1,2-Man-α-1,2-Man and some Man-α-1,3-Man-α-1,2-Man) were prepared by acetolysis of bakers' yeast mannan according to the procedures of Lee and Ballou [4]; α-1,3-mannobiose and α-1,3-mannotriose (Man-α-1,3-Man-α-1,3-Man) were prepared by a similar procedure from the acidic extracellular polysaccharide of *C. laurentii* which contains an α-1,3 linked mannosyl-backbone [5]. The dissacharides were purified by electrophoresis in 0.04 M borate buffer (ph 9.2) and their structures were confirmed by lead tetraacetate degradation [6]. All other chemicals used in these experiments were of the highest grade commercially available.

* All sugars are of the *D* configuration unless otherwise noted.

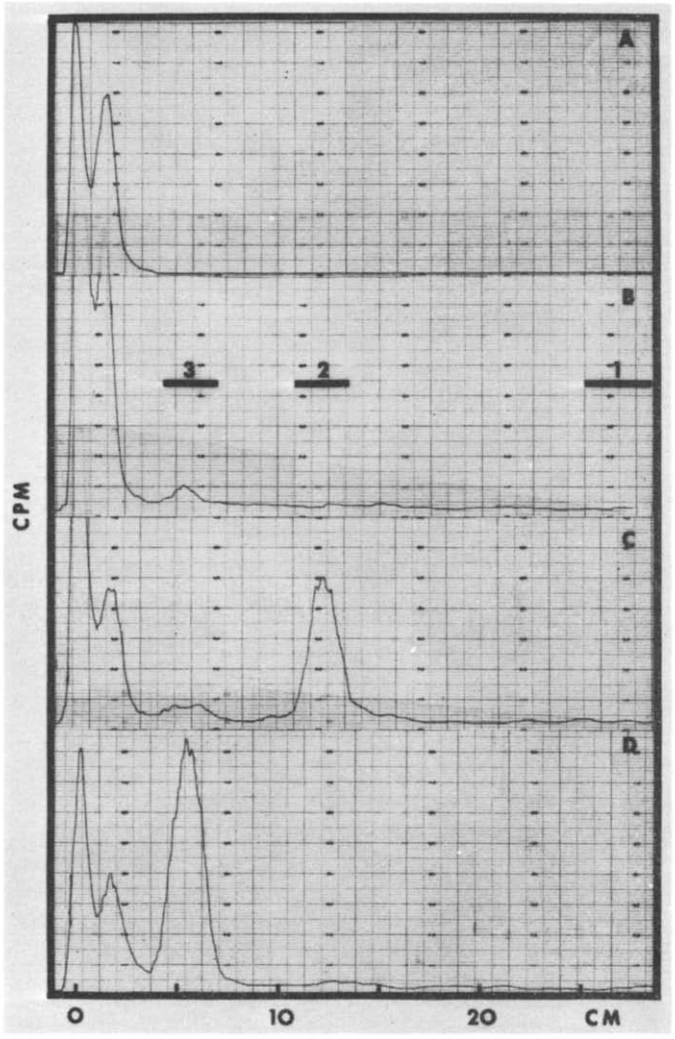

Fig. 1. Transfer of mannose from GDP-^{14}C-mannose to mannose (C) and α-1,2-mannobiose (D). Reactions were carried out as described in the text and the reaction products were separated by descending chromatography for 48 hr in Solvent A. (A) shows the 0 time control and (B) a two hour incubation mixture without acceptor. Unreacted GDP-mannose streaks near the origin to give two peaks. (C) shows the formation of mannobiose and (D) the formation of mannotriose. 1, mannobiose; 2, mannobiose; 3, mannotriose.

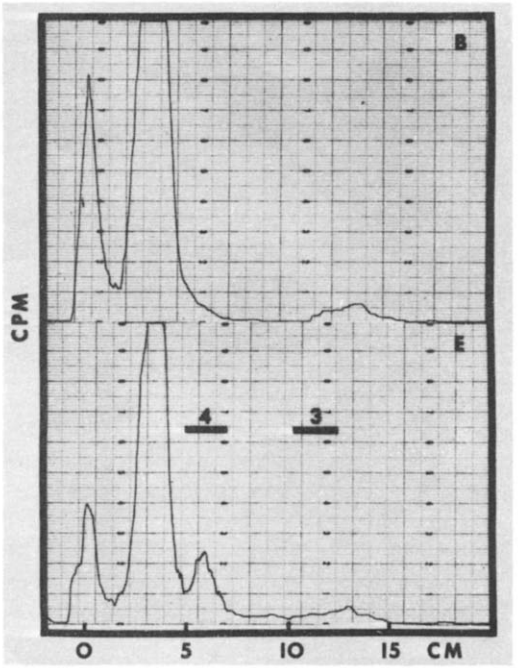

Fig. 2. Transfer of mannose from GDP-^{14}C-mannose to α-1,3-mannotriose (E). Reaction conditions were as described in fig. 1 except that chromatography was carried out for 96 hr. (B) shows a two hour incubation mixture in the absence of acceptor. 3, mannotriose; 4, mannotetraose.

3. Results

Particulate enzyme preparations from *C. laurentii* in reaction mixtures containing GDP-^{14}C-mannose, Mn^{++}, and either mannose, α-1,2-mannobiose, or α-1,2-mannotriose as acceptors catalyze the formation of radioactive products. These products are not formed in control reaction mixtures that do not contain the above acceptors (figs. 1 and 2). The reaction products have chromatographic mobilities identical to mannobiose, mannotriose, and mannotetraose respectively in solvents A and B. They are completely degraded by emulsin (which contains an exo-α-mannosidase [4,7]), to yield mannose as the only radioactive product. The mannosyl residues that originate from GDP-^{14}C-mannose are linked as non-reducing termini to the saccharide acceptors according to the following evidence: when the radioactive oligosaccharides are first reduced with sodium borohydride and then hydrolyzed with 1 N HCl for 3 hr at 100°, mannose is the only radioactive product liberated. The radioactive oligosaccharides have molecular weights corresponding to di-,

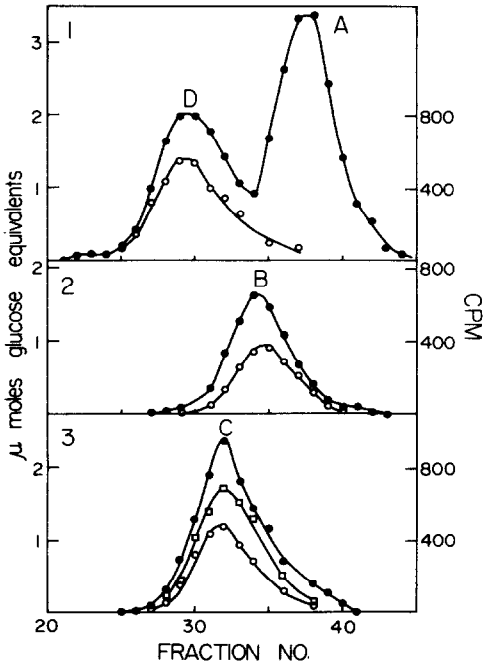

Fig. 3. Gel filtration of ^{14}C-mannobiose, (2, o—o), ^{14}C-mannotriose (3, o—o), ^{14}C-mannotetraose (1, o—o) and ^{14}C-xylosyl-(mannose)$_2$ (3, □—□) through a calibrated Sephadex G-15 column (2 X 27 cm). Radioactive mannosyl oligosaccharides were isolated from reaction mixtures no. C, D and E, (fig. 1 and 2); ^{14}C-xylosyl-(mannose)$_2$ was prepared from UDP-^{14}C-xylose and α-1,3-mannobiose and isolated after chromatography in solvent B. Unlabeled standards (●—●) were assayed with phenol-sulfuric acid [8]. A, glucose; B, maltose; C, maltotriose; D, maltotetraose.

tri-, and tetrasaccharides respectively as judged by gel filtration through a calibrated Sephadex G-15 column (fig. 3). The radioactive disaccharide has the same electrophoretic mobolity in 0.04 M borate buffer as authentic α-1,2-mannobiose (fig. 4). The following saccharides do not act as acceptors for the transfer of mannose from GDP-mannose: glucose, galactose, xylose, L-arabinose, α-1,3-mannobiose and α-1,3-mannotriose.

When UDP-^{14}C-xylose is used as the glycosyl donor in similar reaction mixtures containing α-1,3-mannobiose as the acceptor, the xylosyl residue that is transferred is also bound as non-reducing

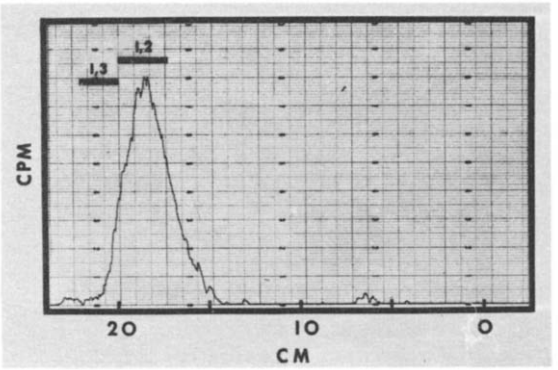

Fig. 4. Electrophoresis of ^{14}C-mannobiose isolated from reaction C (fig. 1) in 0.04 M borate buffer, pH 9.2, for 7 hr at 30 volts/cm. 1,2 is α-1,2-mannobiose; 1,3 is α-1,3-mannobiose.

terminus in the product, an oligosaccharide that has the chromatographic mobility of a trisaccharide in solvents A and B. This product has the molecular weight of a trisaccharide as judged by gel filtration through a Sephadex G-15 column (fig. 3). Xylose is the sole radioactive product liberated upon hydrolysis in 1 N HCl at 100° for 3 hr. When the trisaccharide is treated with α-mannosidase, the major radioactive product has the chromatographic mobility of a disaccharide in solvent B. Reduction of the trisaccharide with sodium borohydride followed by acid hydrolysis liberated xylose as the sole radioactive product, which demonstrates that the ^{14}C-xylosyl residue is linked as non-reducing terminus to the acceptor disaccharide. The following saccharides do not act as acceptors for the transfer of xylose from UDP-xylose: mannose, glucose, galactose, xylose, *L*-arabinose and α-1,2-mannobiose.

UDP-^{14}C-galactose and UDP-^{14}C-glucuronic acid do not serve as glycosyl donors when assayed under the same conditions used to demonstrate mannosyl and xylosyl transfer to acceptors.

4. Discussion

The results presented in this paper demonstrate that mannose, α-1,2-mannobiose, and α-1,2-mannotriose act as acceptors for the incorporation of mannose from GDP-^{14}C-mannose, and that α-1,3-mannobiose acts as an acceptor for the incorporation of

xylose from UDP-^{14}C-xylose. In these reactions only one glycosyl unit is transferred to the added acceptor. Sequential addition of a second glycosyl unit probably does not occur due to the low concentration of radioactive product in the reaction mixtures. Cleavage by α-mannosidase indicates that the newly formed mannosyl linkages are of the α-configuration, but the positions of the linkages have yet to be determined.

The release of ^{14}C-xylosyl-mannose from ^{14}C-xylosyl-(mannose)$_2$ by α-mannosidase suggests that xylose is linked to the mannose rather than to the mannosyl unit of the disaccharide acceptor.

Since α-1,2-mannobiose, the presumed product formed from mannose and GDP-mannose, acts as an acceptor for the addition of a third mannosyl residue to the non-reducing end, these transfer reaction may represent the initial steps in heteropolysaccharide biosynthesis.

By following the approach indicated in this communication – stepwise addition of monosaccharides to oligosaccharides of increasing complexity – we think that it may be possible to elucidate the structure and the biosynthesis of the cell wall heteropolysaccharides of *C. laurentii.*

Acknowledgements

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