Xylosyltransferases in Cryptococcus laurentii*

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SUMMARY

A particle-bound enzyme preparation from the fungus imperfectus, Cryptococcus laurentii, contains at least two different xylosyltransferases. One of these catalyzes the transfer of xylosyl units from UDP-xylose to endogenous cell envelope glycoproteins. The enzyme requires the disaccharide 3-O-α-D-mannosyl-β-D-mannose, or the same structure at the terminal nonreducing end of an oligosaccharide as an acceptor. The xylosyl residue is transferred to the pentultimate mannosyl at the nonreducing end of the acceptor with the formation of a β-1,2 linkage. The product, 3-O-α-D-mannosyl-(2-O-β-D-xylosyl)-β-D-[14C]mannose, was synthesized, reduced with sodium borohydride, oxidized with periodate, again reduced, and then hydrolyzed. This procedure led to the formation of [14C]erythritol confirming the structure of the product.

The xylosyltransferase has a pH optimum of 6.0 to 6.5. The apparent K∞ values for UDP-xylose and mannobiose are 1.4 mM and 19 mM, respectively. These properties, as well as the nature of the product formed, stability of the enzyme at 38°, and inhibition by nucleotides, clearly differentiate between this enzyme and a second xylosyltransferase that has been described for C. laurentii (COHEN, A., AND FEINGOLD, D. S. (1967) Biochemistry 6, 2933).

EXPERIMENTAL PROCEDURES

Materials

Man-α-mannose, Man-2-Man-α-mannose, Man-3 Man-2 Man-2-mannose, Man-6-mannose, Man-3-mannose, and Man-3 Man-3-mannose were prepared as previously described (2, 4). Man-3-mannose and Man-6-mannose were also prepared by acid reversion in 6 N HCl (5). Xylosyl acceptor prepared from C. laurentii acidic polysaccharide was a gift from Dr. D. S. Feingold, University of Pittsburgh, School of Medicine (Acceptor B, see Reference 8). α-Threose was prepared from α-galactose by lead tetraacetate oxidation and reduced with sodium borohydride to yield α-threitol (6). All other chemicals were obtained from commercial sources.

Sweet almond Emulsin (Worthington) was used as a source of both α-mannosidase and β-xylosidase (7, 8). The α-mannosidase has been purified from this source and partially characterized (7). The β-xylosidase has a pH optimum of 5.5 to 6.0. One milligram of Emulsin will hydrolyze 0.6 μmole of β-(p-nitrophenyl) β-xylopyranoside per hour at pH 5.5, 25°, at a substrate concentration of 9 mM. The enzyme hydrolyzes β-Xyl-CH₂ at a very slow rate but does not hydrolyze α-Xyl-CH₂. At a substrate concentration of 9 mM, β-Xyl-CH₂ at 0.2 m inhibits the enzyme 25%. α-Xyl-CH₂ does not inhibit the xylosidase under the same conditions.

Enzyme Preparation

C. laurentii var. flavescens (NRRL Y-1401) was grown and harvested as described previously (9). The standard medium consisted of 0.1% urea, 0.1% KH₂PO₄, 0.05% MgSO₄, 5H₂O, and 0.2 mg per liter of thiamine-HCl. Glucose (2% w/v) was added to the standard medium. The preparation of cell-free extracts and the isolation of the particulate enzyme fraction followed procedures previously described (2). All operations were carried out at 0-4°. Log phase cells (optical density at 400 nm, 0.7) were harvested by centrifugation at 12,000 x g for 15 min, washed with 10 volumes of 1% NaCl, 10 volumes of distilled water, and 10 volumes of 0.1 M Tris-HCl buffer, pH 7.3, 1 mM in second enzyme are presented as well as an analysis of the linkage formed.

1 The abbreviations used are: Man-2-mannose, 2-O-α-D-mannosyl-α-D-mannose; Man-3-mannose, 3-O-α-D-mannosyl-α-D-mannose; Man-6-mannose, 6-O-α-D-mannosyl-α-D-mannose; Man-3 Man-2-mannose, (2-O-α-D-mannosyl)-β-D-mannose; Man-3 Man-3-mannose, (3-O-α-D-mannosyl)-β-D-mannose; Man-3 Man-2 Man-2-mannose, 3-O-α-D-mannosyl-(2-O-α-D-mannosyl)-β-D-mannose; Man-CH₁, methyl-α-D-mannopyranoside; α-Xyl-CH₂, methyl-α-D-xylopyranoside; β-Xyl-CH₁, methyl-β-D-xylopyranoside.
reduced glutathione and EDTA. The washed residue was resuspended in approximately 1 volume of the same buffer and was disrupted by ultrasonic treatment in a Branson Sonifier (model 125) for 6 min at an output of 11 amp.

The cell debris was removed by centrifugation at 12,000 × g for 15 min; the particulate fraction was then isolated by centrifugation at 105,000 × g for 30 min. The pellet so obtained was resuspended in approximately 10 volumes of the above buffer and again centrifuged. This step was repeated once. The particulate material separates into three distinct layers. The brown middle layer contains all of the xylosyltransferase activity and was carefully isolated and resuspended in 3 volumes of the same buffer. The final pH of the enzyme suspension is approximately 6.3 to 6.5 and the buffering capacity of the protein maintains this pH in reaction mixtures. The enzyme retains activity for at least 2 weeks at 4°C.

**Analytical Methods**

Protein was determined by the procedure of Lowry et al. (10). Radioactivity was measured in a gas flow counter (Nuclear Chicago). Paper chromatograms and electrophoretograms were analyzed for radioactivity with a Nuclear Chicago Actigraph III or by autoradiography.

**Chromatography and Electrophoresis**

Paper chromatography was carried out on Whatman No. 1 or No. 3MM paper. The samples used were: Solvent 1, 1 propyl-ethyl acetate-water, 7:1:2; Solvent 2, ethyl acetate-pyridine-water, 8:2:1; Solvent 3, ethyl acetate-acetic acid-water, 3:1:1; Solvent 4, 95% ethanol-1 M ammonium acetate, 7:3; Solvent 5, 1-butanol saturated with water.

Electrophoresis was carried out on Whatman No. 1 paper at 30 volts per cm for 8 hours in Buffer 1 (0.04 M sodium borate, pH 9.2), or on Whatman No. 3MM paper at 30 volts per cm for 40 min in Buffer 2 (0.1 M sodium molybdate adjusted to pH 5.0 with sulfuric acid) (11). Carbohydrates were detected with p-anisidine phthalate (12) or by silver nitrate-acetone spray (13). Protein was determined by the procedure of Lowry et al. (10).

**Enzyme Assays**

**Assay 1**—This assay was used to measure xylosyl transfer from UDP-[14C]xylose to endogenous acceptor. Reaction mixtures contained UDP-[14C]xylose (0.05 μCi; 172 mCi per mmole) and enzyme (0.5 mg of protein) in a total volume of 0.05 ml of Tris-HCl buffer, pH 6.3 to 6.5. The mixture was incubated at 25°C and the reaction was terminated by applying the mixture to strips (4 × 10 cm) of Whatman No. DE81 anion exchange paper. The paper was eluted with 1 ml of water onto planchets. The eluates were dried under reduced pressure and the eluted radioactivity was determined in a gas flow counter.

**Reduction with NaBH₄**

Reductions were carried out by adding 0.05 ml of 0.5 M NaBH₄ to a solution of radioactive sugars in 0.1 ml of water. The reaction mixtures were incubated at room temperature for 16 hours, then treated with cation exchange resin. Boric acid was removed by repeated evaporation in the presence of methanol.

**Preparation of ¹⁴C-Labeled Acceptors**

[¹⁴C]Man-3-mannose and Man-3-[¹⁴C]mannose were synthesized using a specific Mg²⁺-dependent mannosyltransferase present in *C. laurenti* middle layer (4). The first of these acceptors was prepared in reaction mixtures containing mannose (50 μmol), GDP-[¹⁴C]mannose (0.5 μCi; 151 mCi per mmole), MgCl₂ (6 μmol), and middle layer enzyme (5 mg) in a total volume of 0.4 ml of 0.1 M Tris-HCl buffer, pH 7.5. The reaction mixture was incubated for 20 hours at 25°C. The particulate enzyme was removed by centrifugation at 10,000 × g for 10 min, washed once with 0.2 ml of water, and supernatant and wash were combined. The product was isolated after paper chromatography in Solvent 1. Yield of disaccharide based on the amount of [¹⁴C]mannosyl transferred are 60%. Disaccharide containing [¹⁴C]-labeled mannose at the reducing end was prepared in reaction mixtures containing [¹⁴C]mannose (0.25 μmol; 10 mCi per mmole), GDP-mannose (0.5 μmol), MgCl₂ (6 μmol), and middle layer enzyme (5 mg of protein) in a total volume of 0.4 ml of 0.1 M Tris-HCl buffer, pH 7.5. The reaction mixture was incubated at 25°C for 20 hours. Disaccharide was isolated as above in yields of 2% based on the incorporation of [¹⁴C]mannose into product.

The [¹⁴C]-labeled disaccharides were characterized by chromatography with authentic Man-3-mannose in Solvents 1, 2, and 3, by electrophoresis in Buffer 1, and by hydrolysis with α-mannosidase (Emulsin) in ammonium acetate buffer, pH 5.0. These conditions are sufficient to identify all four possible α-D-mannopyranosyl-β-D-mannose disaccharides (4).

The disaccharides (1000 cpm) were reduced with sodium borohydride and then hydrolyzed in 2 N HCl for 3 hours at 100°C. Chromatography of the hydrolysates in Solvent 2 revealed that all of the label recovered from reduced Man-3-[³²P]mannose was [¹⁴C]mannitol, whereas the hydrolysate from [¹⁴C]Man-3-mannitol contained only labeled mannose.

**RESULTS**

**Xylosyl Transfer to 2,0-D-Mannosyl-β-D-mannose**

Paper chromatography of reaction mixtures containing particulate "middle layer" enzyme from *C. laurenti*, UDP-[¹⁴C]xylose, and Man-3-mannose reveals the formation of a new radioactive product (Fig. 1). The product has a chromatographic mobility comparable to a trisaccharide in Solvents 1, 2, and 3 and has the molecular weight of a trisaccharide as judged by gel filtration through a calibrated Sephadex G-15 column (13). The rate of the transferase reaction is linear with time for at least 90 min (Fig. 2) and is proportional to enzyme concentration (Fig. 3).

The formation of the radioactive trisaccharide has an absolute
FIG. 1 (left). Xylosyl transfer from UDP-[14C]xylose to Man-3-mannose. Reaction Mixture A contained enzyme (0.5 mg of protein) and UDP-[14C]xylose (0.05 μCi; 172 mCi per mmole) in a total volume of 0.04 ml of 0.05 M Tris-HCl, pH 6.3 to 6.5. Reaction Mixture B, in addition, contained 8 mM Man-3-mannose. Reactions were incubated for 4 hours. The figures show radioactive strip scans obtained after ascending chromatography in Solvent 1. Full scale deflection equals 1000 cpm. The standards are (1) UDP-xylose, (2) Man-3-Man-2-mannose, (3) Man-α-mannose.

In order to characterize the xylose-containing product, labeled trisaccharides were prepared. One trisaccharide was prepared from UDP-[14C]xylose and [14C]Man-3-mannose. Reaction mixtures contained UDP-[14C]xylose (0.5 μCi; 172 mCi per mmole), [14C]Man-3-mannose (0.25 μCi; 151 mCi per mmole), and enzyme (5 mg of protein) in a total volume of 0.4 ml of 0.05 M Tris-HCl, pH 6.3 to 6.5. After 24 hours of incubation, the product was isolated by chromatography in Solvent 1 (Reaction A).

A second trisaccharide, labeled with [14C]xylose, was prepared in standard reaction mixtures (see "Experimental Procedures; Assay 3") incubated for 4 hours. The reaction product was separated by chromatography in Solvent 1 and eluted from the paper (Reaction B).

Incubation of the labeled trisaccharides with Emulsin (1 mg; a crude enzyme preparation that contains both α-mannosidase and β-xylosidase activity) in 0.2 ml of ammonium acetate buffer, pH 5.0, for 16 hours resulted in the liberation of labeled sugars, either [14C]xylose (Reaction B, Fig. 4) or both [14C]xylose and [14C]mannose (Reaction A, Fig. 4). In the experiment shown in Fig. 4B, xylose was quantitatively liberated from the material migrating slower than xylose by again incubating it with Emulsin. These results indicate that the xylose linkage is of the β configuration.

Incubation of the labeled trisaccharides with Emulsin was carried out as above, except that the reaction mixtures contained 0.5 mM β-Xyl-CH₂ to inhibit the β-xylosidase activity. These results indicate that α-mannosidase hydrolyzes the terminal nonreducing mannosyl residue in the trisaccharide resulting in the formation of a radioactive xylosyl-mannose disaccharide (Fig. 5).

Sodium borohydride reduction of trisaccharide containing only labeled xylose, followed by hydrolysis in 1 N HCl for 2 hours at 100°C and chromatography in Solvent 2, results in the formation of labeled xylose but not xylitol.

This evidence demonstrates that the xylose residue is transferred to the mannose at the reducing end of Man-3-mannose resulting in the formation of a branched trisaccharide.

Determination of Linkage

Trisaccharide containing [3-14C]mannose at the reducing end was prepared in reaction mixtures containing Man-3-[3-14C]-mannose (0.03 nmole; 10 mCi per mmole), UDP-xylose (0.5 μmoles), and middle layer enzyme (5 mg of protein) in a total volume of 0.3 ml of 0.05 M Tris-HCl, pH 6.3 to 6.5. Reaction mixtures were incubated at 25°C for 16 hours and the labeled product (0.32 nmole) was isolated after chromatography of the reaction mixture in Solvent 1. The isolated trisaccharide was reduced with sodium borohydride and an aliquot was hydrolyzed in 1 N HCl for 3 hours at 100°C. Paper chromatography in Solvent 2 revealed that the only radioactive product was mannitol.

The remainder of the reduced trisaccharide (0.3 nmole) was oxidized with sodium periodate (20 μmoles) at room temperature for 6 hours in a volume of 0.4 ml. Ethylene glycol (25 μmoles) was added to destroy excess periodate, followed by the addition of 30 mg of barium carbonate. The precipitate was
FIG. 4 (left). Hydrolysis of [14C]xylosyl- and [14C]mannosyl-labeled trisaccharide (A) and [14C]xylose-labeled trisaccharide (B) with Emulsin. Reaction mixtures containing labeled trisaccharide and Emulsin (1 mg) in a total volume of 0.2 ml of 0.5 M ammonium acetate buffer, pH 5.0, were incubated for 16 hours at 25°. The figures show radioactive strip scans after chromatography of the reaction mixtures in Solvent 1. Full scale deflection equals 1000 cpm. The standards are (1) unhydrolyzed trisaccharide, (2) d-mannose, and (3) d-xylose. The material under the unidentified peak in B when eluted and treated as above with Emulsin is quantitatively hydrolyzed to yield [14C]xylose.

Fig. 5 (center). Hydrolysis of [14C]xylosyl- and [14C]mannosyl-labeled trisaccharide (A) and [14C]xylosyl-labeled trisaccharide (B) with α-mannosidase. Reaction mixtures were as described except that, they contained, in addition, 0.5 M β-Xyl-CH₃ to inhibit the β-xylosidase activity of Emulsin. The figures show radioactive strip scans after chromatography of the reaction mixtures in Solvent 1. Full scale deflection equals 1000 cpm. The standards are (1) unhydrolyzed trisaccharide, (2) d-mannose, (3) d-xylose. The material under the unidentified peak in A and B, when eluted and hydrolyzed in 1 N HCl for 2 hours at 100°, yields only [14C]xylose after chromatography in Solvents 1 and 2.

Fig. 6 (right). Chromatography in Solvent 5 (A) and electrophoresis in Buffer 2 (B) of [14C]erythritol isolated after periodate degradation of reduced trisaccharide labeled with [14C]mannitol. The standards are (1) mannitol, (2) α-threitol, (3) erythritol, and (4) glycerol. Full scale deflection on the radioactive strip scan equals 300 cpm.

FIG. 7. Procedure for the degradation of trisaccharide synthesized from UDP-xylose and Man-3-[3-14C]mannose.

removal by centrifugation and washed three times with water; supernatant and washes were pooled and evaporated under reduced pressure. The residue was dissolved in 0.2 ml of water, reduced with sodium borohydride, and hydrolyzed in 1 N HCl at 100° for 2 hours. The major radioactive spot that could be detected after chromatography in Solvent 5 had the chromatographic mobility of threitol and erythritol (Fig. 6A). Electrophoresis of this material in Buffer 2, which clearly separates both tetritols, showed that the product was erythritol (Fig. 6B). An identical degradation of the disaccharide Man-3-[3-14C]-mannitol followed by chromatography in Solvent 5 gave [14C]-glycerol as the major product. Since only a [3-14C]mannitol residue substituted at both carbon 2 and carbon 3 can yield [14C]erythritol upon periodate oxidation followed by reduction, the xylosyl linkage must be β-1,2. (See Fig. 7.) This result is also consistent with the observation that both the trisaccharide and the xylosyl-mannose disaccharide produced therefrom by α-mannosidase treatment were resistant to short time oxidation with lead tetracetate in the procedure of Charlson and Perlin (14). The structure of the trisaccharide must therefore be 3-O-α-β-mannosyl-(2-O-β-α-xylosyl)-α-mannose.

**Xylosyl Transfer to Endogenous Acceptor**

In reaction mixtures containing only middle layer enzyme and UDP-[14C]xylose, [14C]xylose is transferred to an insoluble endogenous acceptor. The rate of xylosyl transfer to endogenous acceptor is low but is linear with time for at least 8 hours (Fig. 4) except that they contained, in addition, 0.5 M β-Xyl-CH₃ to inhibit the β-xylosidase activity of Emulsin. The figures show radioactive strip scans after chromatography of the reaction mixtures in Solvent 1. Full scale deflection equals 1000 cpm. The standards are (1) unhydrolyzed trisaccharide, (2) d-mannose, (3) d-xylose. The material under the unidentified peak in A and B, when eluted and hydrolyzed in 1 N HCl for 2 hours at 100°, yields only [14C]xylose after chromatography in Solvents 1 and 2.

Fig. 5 (center). Hydrolysis of [14C]xylosyl- and [14C]mannosyl-labeled trisaccharide (A) and [14C]xylosyl-labeled trisaccharide (B) with α-mannosidase. Reaction mixtures were as described except that, they contained, in addition, 0.5 M β-Xyl-CH₃ to inhibit the β-xylosidase activity of Emulsin. The figures show radioactive strip scans after chromatography of the reaction mixtures in Solvent 1. Full scale deflection equals 1000 cpm. The standards are (1) unhydrolyzed trisaccharide, (2) d-mannose, (3) d-xylose. The material under the unidentified peak in A and B, when eluted and hydrolyzed in 1 N HCl for 2 hours at 100°, yields only [14C]xylose after chromatography in Solvents 1 and 2.

Fig. 6 (right). Chromatography in Solvent 5 (A) and electrophoresis in Buffer 2 (B) of [14C]erythritol isolated after periodate degradation of reduced trisaccharide labeled with [3-14C]mannitol. The standards are (1) mannitol, (2) α-threitol, (3) erythritol, and (4) glycerol. Full scale deflection on the radioactive strip scan equals 300 cpm.

8). The rate of the reaction is practically unaffected by the addition of MgCl₂, MnCl₂, or EDTA at concentrations of 10 mM or by GDP-mannose at a concentration of 2 mM to the reaction mixtures. A significant stimulation of xylosyl transfer, however, is noted in reaction mixtures containing both GDP-mannose (2 mM) and MgCl₂ (10 mM) (Fig. 8). This activation could be due to one of two possible mechanisms: (a) stimulation of the xylosyltransferase by the combination of GDP-mannose and MgCl₂; or (b) the synthesis of additional acceptor sites on the endogenous polymer. The latter is possible, because the enzyme preparation contains a magnesium ion-dependent mannosyl transferase (2, 4).
The reaction mixture was incubated for 16 hours at 25 °C, followed by chromatography in Solvent 1, indicated that the enzyme previously incubated with both GDP-mannose and MgCl₂ showed activation of the xylosyl transfer reaction (Table I). This indicates incorporation of d-mannose into endogenous polymer with the formation of new acceptor sites for the xylosyltransferase.

Analysis of Endogenous Product

In order to obtain sufficient amounts of ¹⁴C-labeled product for analysis the following reaction mixture was prepared: middle layer enzyme (7.5 mg), UDP-[¹⁴C]xylose (0.25 μCi; 172 mCi per mmole) in a total volume of 0.2 ml of 0.1 M Tris-HCl buffer, pH 7.0, was incubated at 25 °C for 16 hours. In this reaction mixture, 81% of the counts incorporated into high molecular weight polymer were found in the soluble fraction of the reaction mixture and were quantitatively precipitated by the addition of cetavlon as above. To the combination of supernatant and first wash were added 2 mg of extracellular acidic polysaccharide in 0.1 ml of water. Cetavlon (0.05 ml of a 2.5% solution, w/v) was added to precipitate acidic polysaccharides and after 2 hours at 0 °C, the insoluble material was collected by centrifugation. The precipitate was washed with 0.2 ml of water, dried on a planchet, and counted in a gas flow counter. No counts were present in the cetavlon salt of the acidic polysaccharide; further addition of cetavlon to the supernatant did not cause additional precipitation.

The insoluble product from above (91,000 cpm) was resuspended in 0.3 ml of water and dissolved by the addition of 0.05 ml of 10% (w/v) sodium dodecyl sulfate. A small amount of insoluble material was removed by centrifugation at 10,000 × g for 15 min. All of the counts remained in the supernatant. Three volumes of 95% ethanol were added to the solution and the precipitate was collected by centrifugation at 10,000 × g for 15 min. The precipitate was washed twice with 0.3 ml of 95% ethanol. A total of 32,500 cpm (58%) was recovered in the ethanol precipitate. The water-insoluble precipitate was resuspended in 0.5 ml of water, 1 mg of Pronase (Calbiochem) was added, and the mixture was incubated at 25 °C for 16 hours. Additional Pronase (1 mg) was added and the incubation was continued for 24 hours. Insoluble material was removed by centrifugation, and to the supernatant, 4 volumes of 95% ethanol were added. After 2 hours at 0 °C the insoluble material was collected by centrifugation. The precipitate (33,000 cpm, 36%) was dissolved in 0.5 ml of water and 0.05 ml of 2.5% cetavlon was added to the solution. After 2 hours at 0 °C the precipitate was removed by centrifugation and the ¹⁴C counts in both the precipitate and the supernatant were determined. All of the counts (32,900) were found in the supernatant, indicating that the endogenous polymer labeled with [¹⁴C]xylose was a neutral polysaccharide devoid of uranic acid. An aliquot of the soluble product (2000 cpm) was applied to a Sephadex G-100 column (2 × 25 cm). All of the radioactivity was found in the void volume of the column.

Hydrolysis of the labeled polysaccharide in 1 N HCl for 2 hours at 100 °C, followed by chromatography in Solvent 1, indicated that all of the counts could be accounted for as [¹⁴C]xylose and that the polymer contained only mannose, galactose, and xylose as judged after p-amidinophenylthalamate spray.

Similar results were obtained from the [¹⁴C]xylose-labeled product formed in the absence of added GDP-mannose and MgCl₂, except that the total amount of label incorporated was much less.

A control reaction mixture containing middle layer enzyme (2.5 mg), 0.3 mg of Acceptor B prepared from acidic polysaccharide (see Reference 3), and UDP-[¹⁴C]xylose (0.25 μCi; 172 mCi per mmole) in a total volume of 0.2 ml of 0.1 M Tris-HCl buffer, pH 7.0, was incubated at 25 °C for 16 hours. In this reaction mixture, 81% of the counts incorporated into high molecular weight polymer were found in the soluble fraction of the reaction mixture and were quantitatively precipitated by the addition of cetavlon as above.

These results show that the xylose incorporated into endogenous acceptor can be differentiated from xylose incorporated into the acidic extracellular polysaccharide. The endogenous product is similar in composition and properties to the neutral cell wall polysaccharide previously isolated from C. lauricis (2). The xylosyl residue incorporated into endogenous acceptor is resistant to hydrolysis by Emulsin. This resistance may be due to steric hindrance since we have observed that a terminal,
Evidence for Two Xylosyltransferases—The xylosyltransferase previously described in C. laurentii (3) catalyzes the transfer of D-xylose from UDP-xylose to the acidic extracellular polysaccharide produced by the organism (Enzyme I). This enzyme was reported to have no transferase activity toward oligosaccharide acceptors.

The xylosyltransferase activities described in this paper are shown by the following criteria to be due to a single enzyme (Enzyme II) that is distinct from the previously reported transferase. The two enzymes differ in kinetic properties and stability, as well as in the nature of the product formed.

Kinetic Properties—The two enzymes differ in \(K_m\), pH optima, and inhibition by nucleoside phosphates. In Fig. 9 the dependence of the reaction rate on UDP-xylose concentration is plotted according to the method of Lineweaver and Burk (16). The apparent \(K_m\) values for UDP-xylose calculated from these data are 0.3 mM for Enzyme I and 1.4 mM for Enzyme II with Man-3-mannose as the acceptor. The rate of xylosyl transfer to endogenous acceptor is too low to give reliable data for determination of \(K_m\).

The two enzymes also differ in pH optima (Fig. 11). Whereas Enzyme I has optimum activity at pH 7.5 to 8.0, the pH optimum for Enzyme II is 6.0 to 6.5. The profile of activity versus pH for Enzyme II is similar when assayed with either Man-3-mannose or endogenous polysaccharide as the acceptor. The rate of xylosyl transfer to endogenous acceptor is too low to give reliable data for determination of \(K_m\).

Stability—The two enzymes differ in stability when incubated at 38°C (Fig. 12). Enzyme I retains full activity at 38°C for at least 30 min, while Enzyme II loses 93% of its activity under the same conditions. Although not presented in Fig. 12, Enzyme II loses 99% transferase activity toward endogenous acceptor when heated at 38°C for 30 min.

Substrate Specificity—Enzyme I requires partially degraded acidic polysaccharide as an acceptor (3). This acceptor can be separated into dialyzable and nondialyzable fractions that have equal acceptor activity on a weight basis. The solubilized and

<table>
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<tr>
<th>Nucleotide (2 mM)</th>
<th>Enzyme I (acetic acid polysaccharide)</th>
<th>Enzyme II</th>
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<tr>
<td>AMP</td>
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<td>0</td>
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<td>31b Man-3- mannose</td>
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These data essentially agree with those of Cohen and Feingold (3). The UTP concentration in these assays was 4 mM.

### Table II
Percentage of inhibition of xylosyltransferases by nucleotides

The standard assays as described under “Experimental Procedures” were used except that reaction mixtures contained in addition the indicated nucleotide. Inhibition is reported as percentage of inhibition relative to controls containing no inhibitor as 100%.

* These data essentially agree with those of Cohen and Feingold (3).

b The UTP concentration in these assays was 4 mM.
The enzyme preparation contains a highly specific, Mg++-linkage. As shown in Fig. 7 and Table I, the incorporation of saccharide is found in the cell envelope of the organism and appears to be part of the glycoprotein complex of this structure. We have demonstrated the presence of a xylosyltransferase in *C. laurertii* that catalyzes the incorporation of d-xylose into the neutral polysaccharide. This enzyme can also utilize d-tri-, and tetrasaccharides that contain a terminal nonreducing Man-3-Man linkage. Yeast mannan with the same linkages in a high molecular weight polymer (18) is not an acceptor. The xylose is transferred to the pentultimate mannosyl residue with the formation of a β-1,2 linkage. The acceptor specificity of the enzyme means that the enzyme is able to differentiate between mannosyl residues on a growing polysaccharide, whether internal, terminal, or pentultimate, and to insert side chains at specific residues. The specificity of the enzyme also appears to prevent activity towards the acidic polysaccharide, even though the latter contains an α-1,3-linked mannose backbone. It may be that the molecular weight of this polysaccharide, or its content of uronic acid, prevents its access to the site of the particular enzyme described in this paper, or that position 2 on otherwise susceptible mannosyl residues is already blocked by other residues, such as glucuronic acid. In fact, the xylosyltransferase that we have described can be differentiated from a second xylosyltransferase that does have acceptor specificity directed toward the acidic polysaccharide. The two enzymes differ in acceptor specificity, *K*ₐ for UDP-xylose, pH optimum, heat stability, and inhibition by nucleotides. All of these criteria indicate the presence of at least two xylosyltransferases. The same criteria indicate that the enzyme that transfers xylose to Man-3-mannose is involved in the synthesis of the cell envelope glycoprotein complex of the organism.

**Acknowledgment**—The skillful technical assistance of Elizabeth Cavey is greatly acknowledged.

**REFERENCES**


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**Fig. 12.** Stability of xylosyltransferases at 38°. Middle layer enzyme was heated at 38°. Aliquots were withdrawn at the times indicated and [3H]xylosyl transfer to Man-3-mannose (○—○) and to acidic polysaccharide (■—■) was measured using standard Assays 2 and 3 as described under “Experimental Procedures.”
Xylosyltransferases in *Cryptococcus laurentii*
John S. Schutzbach and Helmut Ankel


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