Uridine Diphosphate D-Xylose. Acceptor Xylosyltransferase of *Cryptococcus laurentii**

Amikam Cohen and David Sidney Feingold†

ABSTRACT: Extracts of the nonfermentative yeast *Cryptococcus laurentii* catalyze the transfer of D-xylosyl moieties from uridine diphosphate D-xylose to suitable acceptors.

The major portion of the uridine diphosphate D-xylose:acceptor xylosyltransferase, which is particulate bound, can be solubilized with digitonin. Partially dexylosylated capsular polysaccharides of *Cr. laurentii* and *Cryptococcus neoformans* (types A–C) and of the haploid form of *Tremella mesenterica* Fries NRRL Y-6151 function as acceptors. Native *Cr. laurentii* polysaccharide, oligosaccharides of polymerization degree ranging from 2 to 8 prepared by partial acetolysis of the native polysaccharide, β-1,4-linked D-xylodextrins, and cell wall D-mannan isolated from *Saccharomyces cerevisiae* are not acceptors. The uridine diphosphate D-xylose:acceptor xylosyltransferase is inhibited by nucleoside tri- and diphosphates; inhibition is strongest for uridine nucleotides. Cells of *Cr. laurentii* normally contain endogenous inhibitor, since dialysis of the broken cell preparation markedly enhances its transferase activity.

An acidic extracellular polysaccharide which consists of D-mannose, D-xylose, and D-glucuronic acid in the approximate molar ratio 5:2:1 is produced by *Cryptococcus laurentii* var. flavencens NRRL Y-1401 (Abercrombie *et al.*, 1960a; see also Jeanes *et al.*, 1964). Structural studies indicate that the polysaccharide has a backbone of D-mannose residues with D-xylose and D-glucuronic acid end groups. Furthermore the native polysaccharide contains approximately 7% by weight of *O*-acetyl groups (Jeanes *et al.*, 1964).

Cr. laurentii is capable of converting exogenous D-glucose to D-mannosyl and D-glucuronosyl moieties of the acidic polysaccharide with little rearrangement of label, and to D-xylosyl moieties with loss of C_6 (Abercrombie *et al.*, 1960b). This observation together with the isolation of UDPG,[1] UDPXyl, and GDPM from the organism (Ankel *et al.*, 1964) and demonstration of the presence of UDPG dehydrogenase (Ankel *et al.*, 1966) and UDP glucuronate carboxylyase (Ankel and Feingold, 1965) in *Cr. laurentii*, suggests that GDPM, UDPGA, and UDPXyl are terminal glycosyl donors for synthesis of the acidic polysaccharide. In this paper the preparation and some properties of UDP-D-xylose:acceptor xylosyltransferase from *Cr. laurentii* are described.

Experimental Section

Cr. laurentii var. flavescens NRRL-Y-1401 was grown and harvested as described previously (Ankel and Feingold, 1966). UDPG, UDPGA, and UDPXyl, all uniformly labeled with ^{14}C in the glycosyl residue, were prepared from ^{14}C-labeled α-D-glucosyl phosphate as described by Ankel and Feingold (1965). Extracellular polysaccharides of *Cr. laurentii* and *Tremella mesenterica* Fries NRRL Y-6151 were kindly provided by Dr. M. Slodki of Northern Regional Research Laboratories, U. S. Department of Agriculture, Peoria, Ill. Extracellular polysaccharides of *Cryptococcus neoformans* types A–C were a gift from Dr. Jinks Walter of the Graduate School of Public Health, University of Pittsburgh. *Saccharomyces cerevisiae* cell wall mannan was supplied by D. H. Northcote, Department of Biochemistry, The University, Cambridge, England. UDPAra was a gift from Dr. E. F. Neufeld, of the National Institutes of Health. D-Xylodextrins of polymerization degree 2–7 were prepared as described by Whistler and Tu (1951). Acetolysis of *Cr. laurentii* polysaccharide was performed according to the method of Wolfrom *et al.* (1951), and acetolysis products were deacetylated by treatment with sodium methoxide in methanol. The resulting oligosaccharides, which were isolated by elution with 50% ethanol

* From the Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, and the Department of Microbiology, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania. *Received May 15, 1967.* This research was sponsored jointly by Grant GM 08820 from the U. S. Public Health Service and by the U. S. Atomic Energy Commission under contract with the Union Carbide Corp. Some of the results presented here were reported in a preliminary communication (see Cohen and Feingold, 1964).

† Career Development Grant 1 K3-GM-28,296-03 Awardee of the U. S. Public Health Service. Department of Microbiology of the University of Pittsburgh School of Medicine.

[1] Abbreviations used: uridine 5′-(α-D-glucopyranosyl pyrophosphate), UDPG; uridine 5′-(α-D-xylopyranosyl pyrophosphate), UDPXyl; uridine 5′-(β-L-arabinopyranosyl pyrophosphate), UDPAra; uridine 5′-(α-D-glucopyranosyluronic acid pyrophosphate), UDPGA; guanosine 5′-(α-D-mannopyranosyl pyrophosphate), GDPM; nicotinamide–adenine dinucleotide, NAD; reduced NAD, $NADH_2$; uridine triphosphate, UTP; UDP-glucose:NAD oxidoreductase (EC 1.1.1.22), UDPG dehydrogenase.

(after washing with water) from a column of Darco G-60-Celite (Whistler and Durso, 1950) contained either D-mannose or D-glucuronic acid and D-mannose, but no D-xylose. They probably ranged from di- to octasaccharides, as estimated by paper chromatography in solvent A. All other materials used were commercial products.

The following solvent systems were used in ascending paper chromatography on Whatman No. 1 filter paper: solvent A, 1-propanol–ethyl acetate–water (7:2:1); solvent B, 1 M ammonium acetate (pH 7.5)–ethanol (3:7); and solvent C, water-saturated phenol. Protein was estimated by the method of Lowry *et al.* (1951).

Preparation of Acceptor. Acceptor was prepared by partial degradation of *Cr. laurentii* polysaccharide according to the method described by Abercrombie *et al.* (1960a). Aqueous solutions (5%) of the product were autoclaved at 120° for 5 min; this treatment decreased the viscosity of the solution and doubled its acceptor activity. Except when otherwise stated, this solution was used as "acceptor" in all reaction mixtures. The polysaccharide product of the hydrolysis was further resolved into a dialyzable (D) and a nondialyzable (ND) fraction by dialysis of a 5% solution against 800 volumes of water, followed by concentration of the dialysate at 37° to a syrup and subsequent recovery of both the dialysate and retentate by precipitation with 20 volumes of ethanol. Both fractions have similar acceptor activities when used in equal weight-to-volume concentrations. Acceptors were also prepared from the native acidic polysaccharide of *Cr. neoformans*, types A–C, and from *T. mesenterica* polysaccharide by partial hydrolysis and ethanol precipitation as described above.

Assay of Xylosyl Transfer Reaction. ASSAY 1. This assay is based on the difference in the chromatographic mobilities of UDPXyl and xylosyl acceptor in solvent B. Reaction mixtures contained, at 30°: 20 μl of 5% acceptor solution, 20 μl of 10 mM UDPXyl labeled with ^{14}C in the D-xylosyl moiety (sp act. 1.88 mc/mmole), and 20 μl of enzyme solution. At appropriate time intervals samples were inactivated at 100° and resolved by overnight chromatography on Whatman No. 1 paper in solvent B. The appropriate areas at the origin were cut out and counted in a liquid scintillation spectrometer. Controls were run without acceptor and with boiled enyzme.

ASSAY 2. This assay is based on the difference in solubility of UDPXyl and xylosyl acceptor in 75% methanol, 0.1 M in KCl. Samples of 15 μl were taken from a reaction mixture identical with that used in assay 1 and pipetted into 4 ml of 75% methanol, 0.1 M in KCl. The precipitate was collected by centrifugation, dissolved in 0.2 ml of water, and reprecipitated with 4 ml of methanolic KCl solvent; this procedure was repeated and the resulting precipitate was collected by filtration through a solvent-resistant Millipore filter (filter type OH 02500). The filters were further washed with 20 ml of the methanolic KCl, dried, and counted in the liquid scintillation spectrometer.

Definition of Unit of Enzyme Activity. A unit of enzyme activity is defined as that amount of enzyme which transfers 1 mμmole of D-xylose/min at 30°; specific activity is expressed as units per milligram of protein.

Preparation of Cell-Free Extracts. All operations were carried out at 0–4° except where otherwise indicated. Cells were harvested by centrifugation at 12,000*g* for 20 min, resuspended in five volumes of 0.1 M potassium phosphate buffer (pH 7.0), 1 mM in reduced glutathione and EDTA (buffer), and compacted by centrifugation. The washed cells were suspended in an equal volume of the buffer and disrupted as described by Ankel and Feingold (1966). The broken cell suspension was centrifuged at 28,000*g* for 20 min, and the pellet was discarded. The turbid supernatant fluid was then centrifuged at 105,000*g* for 60 min. The supernatant fluid (S1) contained some transferase activity; it was stored at 0–4°. The pellet (P1) was resuspended in ten volumes of buffer and again centrifuged at 105,000*g* for 60 min. The pellet from this centrifugation contained three distinct fractions: an upper white "fluffy" layer, a middle brown layer, and a lower transparent layer. After the supernatant fluid was discarded, the top layer was carefully decanted, and 1 ml of buffer was added to the centrifuge tube. The middle brown layer was suspended in the buffer and decanted into a separate tube, without disturbing the lower layer of the pellet; the lower layer was then suspended in 1 ml of buffer. Each fraction was homogenized in a Teflon-glass homogenizer and tested for activity. Eighty per cent of the total activity was associated with the middle layer (P2), 15% with the bottom layer, and 5% with the top layer.

Solubilization of the Enzyme. Fractions P1 and P2 were each treated as follows. The suspension (5 ml) of particles was mixed with an equal volume of 2% digitonin solution in a 15-ml Rosset cell (Rosset, 1965), treated with the Branson sonifier at an output of 3.5 amp for 10 min, and then centrifuged at 105,000*g* for 60 min. The supernatant fluids (fractions P1s and P2s) were decanted and the pellets were resuspended in buffer. All fractions were tested for activity; in each case 90–95% of the total transferase was found in the soluble fractions (P1s and P2s). When large quantities of particles were used, the total volume treated was scaled up to 100 ml, and treated in a 150-ml Rosset cell at maximum sonifier output. Solubilization of the enzyme increased the total activity. These experiments are summarized in Table I.

Concentration and Partial Purification of Soluble UDPXyl:Acceptor Xylosyltransferase. Fraction S1 was dialyzed against 100 volumes of buffer for 24 hr; the retentate was centrifuged at 105,000*g* for 60 min, and the sediment was discarded. Streptomycin sulfate (20%) was added to the supernatant solution to a final concentration of 1%; after 15 min the precipitate was removed by centrifugation and discarded. The supernatant solution was brought to 40% $(NH_4)_2SO_4$ concentration by addition of a saturated solution of $(NH_4)_2SO_4$ (pH 7). The resultant precipitate was

TABLE I: Fractionation of UDPXyl:Acceptor Xylosyltransferase of *Cr. laurentii*.

Fraction	mg of Protein/ml	Sp Act.[a] (units/mg)	Vol. (ml)	Total Act.[a] (act. units)
Cell-free extract	1.9	2.0	100	380
S1	1.8	0.5	95	85
P1	3.6	8.0	10	288
P2	2.4	17	6	245
P1s	1.7	12	20	408
P2s	1.2	20	12	288

[a] All fractions were dialyzed before assaying.

dissolved in a small volume of buffer. This procedure resulted in a 2.4-fold increase of specific enzymatic activity and yielded a product with specific activity of 1.2 units/mg of protein.

The Effect of Dialysis on the Activity of Enzyme Preparations. Fractions of enzyme preparations were subjected to dialysis against 400 volumes of buffer for 24 hr, and the activity of dialyzed and undialyzed preparations was determined (Table II). A five- to eightfold increase in total and specific activity was observed when crude cell-free extract or fractions S1 and P1 were dialyzed. When fraction P1 (particulate) was washed by suspension in buffer and recentrifugation, the increase in activity was similar to that observed upon dialysis. Subsequent dialysis of the washed preparation did not change its activity markedly.

Incorporation of D-*Xylose from UDPXyl into Acceptor.* The reaction requirements for the transfer of D-xylose from UDPXyl to acceptor are presented in Table III; this system has an absolute requirement for UDPXyl, partially hydrolyzed *Cr. laurentii* polysaccharide (or other acceptors listed below), and enzyme. Fraction P1s was used in this and the following

TABLE II: The Effect of Dialysis on the Activity of Enzyme Preparations.

Enzyme Fraction	Sp Act. (units/mg) Before Dialysis	After Dialysis
Crude	0.3	2.0
P1	0.9	8.0
S1	0.1	0.5
P1 (washed)[a]	6.5	7.2

[a] Activity of P1 (washed) before washing was the same as activity of nondialyzed P1.

TABLE III: Incorporation of D-Xylose into Acceptor.[a]

System	Cpm Incorp
Complete	17,848
−Acceptor	343
−Acceptor + *Cr. laurentii* native polysaccharide	187
−Enzyme	37
−Enzyme + heat inactivated enzyme	45
−UDPXyl + α-D-xylopyranosyl phosphate	23

[a] The complete system contains 23,000 cpm of UDPXyl (12 mc/mM), 16 μg of acceptor, and 10 μl of enzyme preparation (fraction P1s) in a total volume of 30 μl. After incubation the reaction mixture was chromatographed in solvent B, and the area at the origin was cut out and counted in the liquid scintillation spectrometer.

experiments. Native polysaccharide of *Cr. laurentii* does not act as acceptor in this system, nor does α-D-xylosyl phosphate act as donor. Release of UDP was demonstrated by visual inspection of chromatograms (solvent B) prepared from reaction mixtures containing 10 μl of each of the following: UDPXyl (20 μc/10 μmoles per ml), enzyme preparation, and 5% acceptor solution. No detectable UDP was released in systems lacking UDPXyl, acceptor, or enzyme preparation, or when boiled enzyme was used. The newly formed product, like the acceptor, but unlike UDPXyl, was immobile upon paper chromatography in solvent B, insoluble in ethanolic KCl, and formed an insoluble cetavlon salt. The only radioactive compound which was released from the product upon acid hydrolysis (20 hr in 1 N HCl at 100°) or upon partial hydrolysis under the same condition used to prepare the acceptor was identical with carrier D-xylose upon two-dimensional chromatography (solvents A and C) followed by autoradiography.

An experiment was performed to ascertain what percentage of the D-xylose removed from the native polysaccharide could be replaced by the action of D-xylosyltransferase. Acceptor solution (5 μl of 1%) was incubated with 10 μl of 0.2 M ^{14}C-labeled UDPXyl (0.02 mc/mM) and 10 μl of enzyme at pH 6.8. After 14 hr at 30°, a 10-μl sample of reaction mixture was analyzed for incorporation of radioactivity into polysaccharide; 10 μl of 2×10^{-4} M [^{14}C]UDPXyl (40 mc/mM) and 10 μl of fresh enzyme were then added to the remaining reaction mixture. Additional incorporation of radioactivity, determined after 6 more hr at 30°, was less than 2% of that incorporated during the first incubation. Approximately 1 mole of D-xylose was transferred/5500 g of acceptor, which represents replacement of about 20% of the D-xylose removed in preparation of acceptor.

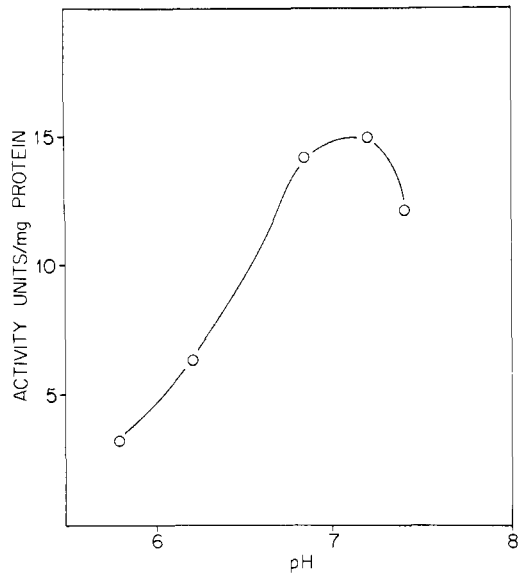

FIGURE 1: Dependence of activity on pH. Experimental conditions were the same as described in the text for assay 1, except that the pH was varied.

Optimum pH. The optimum pH was determined in 0.03 M phosphate buffer between pH 5.6 and 7.4. As can be seen from Figure 1, the pH optimum is about 6.8.

Effect of Enzyme Concentration. The reaction is linear for at least 15 min under the conditions of assay. Activity varies linearly with enzyme concentration (Figure 2).

Effect of Substrate Concentration. The effect of UDPXyl concentration on reaction rate is shown in the lower curve of Figure 3. The apparent K_m value

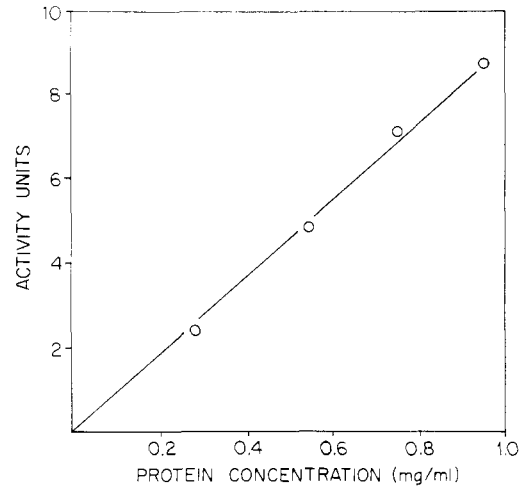

FIGURE 2: Dependence of reaction rate on enzyme concentration. Experimental conditions were the same as those described in the text for assay 1.

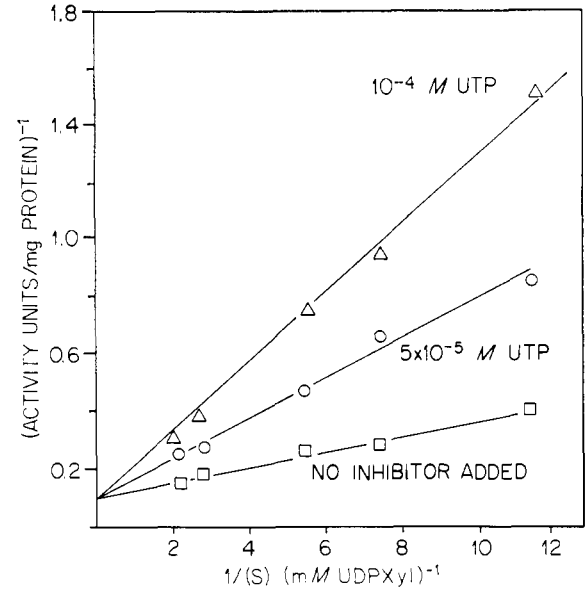

FIGURE 3: Dependence of reaction rate on UDPXyl concentration at different concentrations of UTP. Experimental conditions were the same as those described in the text for assay 1, except that UTP was added to the reaction mixture to give the final concentrations indicated.

at 30°, determined according to the method of Lineweaver and Burk (1934), is 2.6×10^{-4} M. A similar plot of acceptor concentration *vs.* reaction rate is shown in the lower curve of Figure 4. The K_m value calculated for the acceptor is 6.6 mg/ml.

Stability of the Enzyme. Samples of fraction P1s have been kept at 0–4° or frozen at −20° for up to 6 months with little or no loss of activity. One preparation stored at 0–4° for 1 year showed loss of about 20% of the initial activity.

Specificity of UDPXyl-Acceptor Xylosyltransferase. DONOR SPECIFICITY. UDPXyl is the only sugar nucleotide which has been demonstrated as a glycosyl donor with the enzyme. UDPG, UDPGA, GDPM, and α-D-xylopyranosyl phosphate do not act as glycosyl donors in the system described.

ACCEPTOR SPECIFICITY. The D and ND fractions of acceptor prepared by partial hydrolysis of *Cr. laurentii* polysaccharide accept D-xylose at similar rates when used at equal weight-to-volume concentrations. A different partially degraded native *Cr. laurentii* polysaccharide, which functions as an acceptor of D-xylosyl moieties, can be prepared by treatment of the polysaccharide with gut juice of *Helix pomatia* followed by dialysis to remove the D-glucuronic acid, D-mannose, and D-xylose.[2] In addition, polysaccharides of *Cr. neoformans* of serological types A–C, and polysaccharides of *T. mesenterica* act as acceptors when partially degraded under condi-

[2] J. Deshusses and D. S. Feingold, unpublished observations.

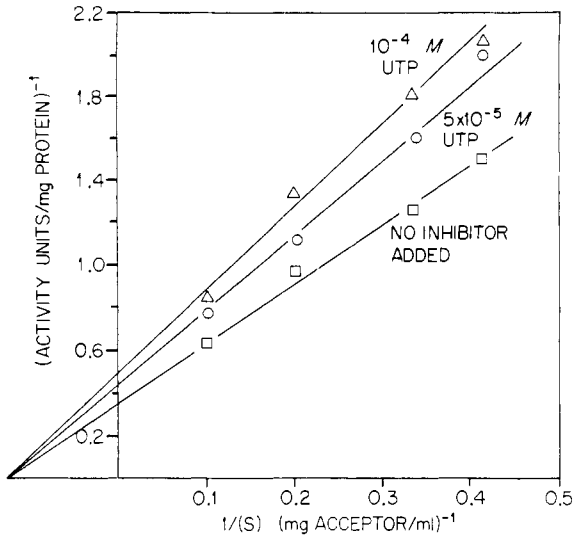

FIGURE 4: Dependence of reaction rate on acceptor concentration at different concentrations of UTP. Experimental conditions were the same as those described in the text for assay 1, except that UTP was added to the reaction mixture to give the final concentration indicated.

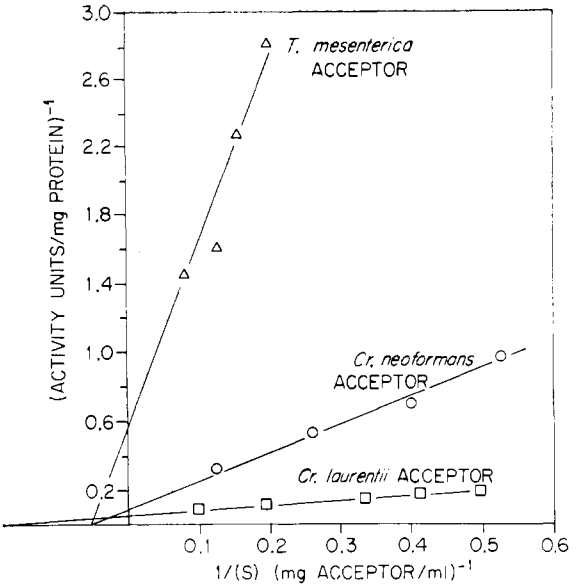

FIGURE 5: Lineweaver–Burk (1934) plot of reaction rates with different acceptors. Experimental conditions were the same as those described in the text, except that acceptor concentration was varied as indicated.

tions used for preparation of acceptor from *Cr. laurentii* polysaccharide. A Lineweaver–Burk (1934) plot for acceptors prepared by resin hydrolysis from polysaccharides of *Cr. laurentii* and *Cr. neoformans* type B and *T. mesenterica* is presented in Figure 5; K_m (6.6 mg/ml) is lowest, and the maximum velocity (V_{max}) (25 units/mg of protein) values are highest for acceptor prepared from *Cr. laurentii* polysaccharide. K_m values for acceptor prepared from *Cr. neoformans* (25 mg/ml) and *T. mesenterica* polysaccharides are identical while the V_{max} value for the *Cr. neoformans* acceptor (10 units/mg of protein) is six times higher than that for acceptor prepared from *T. mesenterica* polysaccharide. There is no significant difference in K_m or V_{max} between acceptors prepared from polysaccharides of *Cr. neoformans* types A–C.

The following compounds do not act as acceptors in this system: native polysaccharides of *Cr. laurentii* and *Cr. neoformans* types A–C and *T. mesenterica*, oligosaccharides prepared from *Cr. laurentii* polysaccharide by acetolysis, *Saccharomyces cerevisiae* cell wall mannan (Northcote and Horne, 1952), and xylodextrins of polymerization degree 2–7.

Inhibitors of UDPXylose: Acceptor Xylosyltransferase. All nucleoside triphosphates that were tested and some nucleoside diphosphates and monophosphates inhibited the reaction. An attempt to determine the degree of inhibition as well as types of inhibition by these compounds is illustrated in Figures 4 and 5 and summarized in Table IV. K_i values were calculated from the Lineweaver–Burk (1934) plots according to the method described by Dixon and Webb (1964).

The most inhibitory of the compounds tested is UTP, with K_i of 2.5×10^{-5} M. Nucleoside diphosphates inhibit less than nucleoside triphosphates with the same base, while nucleoside monophosphates are poorer inhibitors than are homologous nucleoside diphosphates. Although the D-xylosyl donor contains a pyrimidine residue, in general pyrimidine compounds do not inhibit more than purines. Inhibition by nucleotides decreases in the following order: uridine, adenosine, thymidine, cytidine, and guanosine.

In addition to the compounds listed above, UDPAra, UDPG, and to a lesser degree UDPGA, GPDM, and inorganic pyrophosphate inhibit the reaction. All the inhibitors tested were shown to behave competitively with respect to UDPXyl and noncompetitively with respect to the acceptor.

Discussion

The above data indicate that extracts of *Cr. laurentii* catalyze the transfer of D-xylosyl moieties from UDPXyl to a suitable acceptor. The likelihood that the enzyme responsible, UDP-D-xylose:acceptor xylosyltransferase, is involved in the synthesis of the acidic extracellular polysaccharide of *Cr. laurentii* is substantiated by its high specificity for acceptor as well as by the similarity of the product resulting from D-xylosyl transfer to native polysaccharide in chromatographic behavior, precipitability in ethanol, formation of an insoluble cetavlon salt, and lability upon hydrolysis with acidic ion-exchange resin. The probability that the transferase is involved in the synthesis of the acidic extracellular polysaccharide of *Cr. laurentii* is substantiated by isolation of UDPXyl from *Cr. laurentii* (Ankel *et al.*,

TABLE IV: Inhibition of UDPXyl:Acceptor Xylosyltransferase by Nucleotides.

Inhibitor[a]	% Inhibn[b] by 3×10^{-3} M Inhibitor	K_i (UDPXyl) $\times 10^5$	K_i (acceptor) $\times 10^5$
UTP	91	2.4	26
UDP	80	5.8	
UMP	11	500	
ATP	60	5.2	26
ADP	34	540	
AMP	8		
TTP	62	25	
TDP	—		
TMP	7		
CTP	38	72	
CDP	<5		
CMP	<5		
GTP	40	90	
GDP	<5		
GMP	<5		
Inorganic pyrophosphate	8		
UDPAra	60		
UDPG	16		
UDPGA	9		
GDPM	10		

[a] Abbreviations used: UMP, UDP, and UTP, uridine mono-, di-, and triphosphates; AMP, ADP, and ATP, adenosine mono-, di-, and triphosphates; TMP, TDP, and TTP, thymidine mono-, di-, and triphosphates; CMP, CDP, and CTP, cytidine mono-, di-, and triphosphates; GMP, GDP, and GTP, guanosine, mono-, di-, and triphosphates. [b] Experimental conditions are as described in the text. UDPXyl concentration is 3×10^{-3} M; acceptor concentration, 8.4 mg/ml.

1964) as well as demonstration of the pathway which leads to its formation (Ankel and Feingold, 1965; Ankel *et al.*, 1966).

UDP-D-xylose:acceptor xylosyltransferase displays a certain measure of acceptor specificity which seems to require the presence of a branched D-mannan of a particular structure, with available sites for D-xylosyl transfer.

D-Mannan of *S. cerevisiae* cell wall, which has a different structure, is inactive as is a D-mannan prepared from *Cr. laurentii* polysaccharide by Smith degradation (Smith and Umrau, 1959).[2] Native polysaccharides of *Cr. laurentii*, *Cr. neoformans*, and *T. mesenterica* are not acceptors, whereas the partially dexylosylated polysaccharides are. Therefore, the polysaccharide must contain but a limited number of acceptor sites; in the native polysaccharide these probably are saturated with D-xylosyl moieties and unavailable. Partial hydrolysis unblocks some of the sites by removal of the D-xylosyl residues bound to them.

Within a certain range, molecular weight of the acceptor seems to play little or no role; however, low molecular weight oligosaccharides prepared from the native polymer are not acceptors, showing that the acceptor either must be above some minimum molecular or possess a particular structural feature in order to be active.

While the best acceptor for xylosyl transfer by the *Cr. laurentii* enzyme is the partially hydrolyzed polysaccharide produced from the organism, active acceptors can be prepared from the native polysaccharide of the closely related *Cr. neoformans* and from the native polysaccharide of the supposedly unrelated *T. mesenterica*, although both the affinity constant and the V_{max} are least favorable for this substance (Figure 5). Nevertheless, the ability of *Cr. laurentii* enzyme to catalyze the incorporation of D-xylosyl moieties into acceptor prepared from *T. mesenterica*, as well as the ability of extracts of *T. mesenterica* to catalyze the transfer of D-xylosyl moieties from UDPXyl into acceptor prepared from *Cr. laurentii* polysaccharide[3] may indicate a possible taxonomic relationship between the two organisms. This conclusion is supported by the observations of Slodki (1966) of the similarity between hydrolysis products of the polysaccharides of the two organisms and the morphological similarity between *Cr. laurentii* cells and haploid cells of *T. mesenterica*.

Only 20% of the D-xylosyl moieties removed in preparation of acceptor could be replaced by action of the enzyme. Failure to accomplish complete replacement of D-xylose can be explained in a number of ways. The acceptor used, while active, is doubtless not the true physiological acceptor and might only show activity by virtue of similarity of some particular acceptor sites. In addition, the digitonin-solubilized enzyme preparation certainly differs from the native, particulate enzyme which is probably membrane bound in the intact cell.

In general, the type of acceptor active in this system is similar to those involved in the biosynthesis of *Pneumococcus* type I polysaccharide (Mills and Smith, 1962) and of the lipopolysaccharides of Gram-negative bacteria (Horecker, 1966). In all three cases, side-chain residues are incorporated into a preformed backbone. In the synthesis of the *Cr. laurentii* polysaccharide, one can envisage initial formation of a D-mannan from GDPM, followed by incorporation of D-xylosyl and D-glucuronsyluronic acid residues from UDPXyl and UDPGA, respectively, although not necessarily in that order. This aspect of the problem is at present under investigation.

[3] D. S. Feingold, unpublished results.

It was demonstrated previously that UDPG is the major uridine diphosphate sugar of *Cr. laurentii* (Ankel *et al.*,1964). In *Cr. laurentii*, as in other organisms which synthesize UDPXyl, this compound is formed from α-D-glucopyranosyl 1-phosphate as follows.

$$\text{UTP} + \alpha\text{-D-glucopyranosyl phosphate} \rightleftharpoons \text{UDPG} + \text{inorganic pyrophosphate} \quad (1)$$

$$\text{UDPG} + 2\text{NAD} \longrightarrow \text{UDPGA} + 2\text{NADH}_2 \quad (2)$$

$$\text{UDPGA} \longrightarrow \text{UDPXyl} + \text{CO}_2 \quad (3)$$

Since the product of reaction 3 is an allosteric feedback inhibitor of reaction 2 (Ankel *et al.*, 1966) and since the activity of UDPGA carboxy-lyase, which catalyzes reaction 3, is determined by the NAD^+:$NADH_2$ ratio (Ankel and Feingold, 1966), it has been proposed that these separate but related controls serve to regulate the relative concentrations of two of the terminal precursors of polysaccharide synthesis, UDPGA and UDPXyl (Ankel *et al.*, 1966). Inhibition of UDPXyl:acceptor xylosyltransferase by UTP might permit sufficient UDPXyl to accumulate to effectively block dehydrogenase action, resulting in the observed increased concentration of UDPG. The latter substance may be needed by the cell for the synthesis of cell wall structures or of the extracellular D-glucan produced by *Cr. laurentii* under certain conditions (Abercrombie *et al.*, 1960a).

Dialysis of crude extracts of *Cr. laurentii* or of S1 or P1 results in a substantial increase (tenfold) of transferase activity for P1 (Table II). These results indicate the intracellular presence of low molecular weight endogenous inhibitors. Since such inhibitors are present in far greater concentration within the cell, their *in vivo* effect is probably more pronounced. No attempt has been made to identify the nature of the inhibitory substance (or substances).

References

Abercrombie, M. J., Jones, J. K. N., Lock, M. V., Perry, M. B., and Stoodley, R. J. (1960a), *Can. J. Chem. 38*, 1617.

Abercrombie, M. J., Jones, J. K. N., and Perry, M. B. (1960b), *Can. J. Chem. 38*, 2007.

Ankel, H., Ankel, E., Feingold, D. S. (1966), *Biochemistry 5*, 1864.

Ankel, H., Farrell, D. G., and Feingold, D. S. (1964), *Biochim. Biophys. Acta 90*, 397.

Ankel, H., and Feingold, D. S. (1965), *Biochemistry 4*, 2468.

Ankel, H., and Feingold, D. S. (1966), *Biochemistry 5*, 182.

Cohen, A., and Feingold, D. S. (1964), *6th Intern. Congr. Biochem., N. Y.*, VI-19.

Dixon, M., and Webb, E. C. (1964), Enzymes, New York, N. Y., Academic, p 327.

Horecker, B. L. (1966), *Ann. Rev. Microbiol. 20*, 256.

Jeanes, A., Pittsley, J. E., and Watson, P. R. (1964), *J. Appl. Polymer. Sci. 8*, 2005.

Lineweaver, H., and Burk, D. (1934), *J. Am. Chem. Soc. 56*, 658.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.

Mills, G. T., and Smith, E. E. B. (1962), *Biochem. J. 82*, 42.

Northcote, D. H., and Horne, R. W. (1952), *Biochem. J. 51*, 353.

Rosset, T. (1965), *Appl. Microbiol. 13*, 254.

Slodki, M. E. (1966), *Can. J. Microbiol. 12*, 495.

Smith, F., and Umrau, A. M. (1959), *Chem. Ind. (London)*, 881.

Whistler, R. L., and Durso, D. E. (1950), *J. Am. Chem. Soc. 72*, 677.

Whistler, R. L., and Tu, C. C. (1951), *J. Am. Chem. Soc. 73*, 1389.

Wolfrom, M. L., Tyree, J. T., Golkowski, T. T., and O'Neill, A. N. (1951), *J. Am. Chem. Soc. 73*, 4927.