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ENZYMIC DEGRADATION OF YEAST CELL-WALL MANNANS AND GALACTOMANNANS TO POLYMERIC FRAGMENTS CONTAINING α-(1→6)-LINKED D-MANNOPYRANOSE RESIDUES*

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ABSTRACT

The cell-wall D-mannans of *Candida parapsilosis*, *Endomycopsis fibuliger*, *Saccharomyces rouxii*, *Torulopsis apicola* (Hajsig strain), and *Torulopsis bombi* were degraded with an exo α-D-mannosidase from *Arthrobacter* GJM-1 to their α-(1→6)-linked D-mannopyranose main-chains, as demonstrated by p.m.r. spectroscopy. D-Galacto-D-mannans from *Candida lipolytica*, *Torulopsis gropengiesseri*, *Torulopsis lactis-condensi*, *Torulopsis magnoliae*, *and Trichosporon fermentans* could be degraded to polysaccharides containing mainly 6-*O*-linked α-D-mannopyranosyl residues following preferential removal of their enzyme-resistant, D-galactopyranosyl non-reducing end-units with acid. The D-mannans of *Saccharomyces lodderi*, *Citeromyces matritensis*, and *Pichia pastoris* could also be enzymically degraded to polysaccharides containing predominantly α-(1→6)-linked D-mannopyranosyl residues after hydrolysis of most of the β-D-linked residues in their side chains with acid. The exo α-D-mannosidase, as would be expected, produced β-D-mannose on splitting of an α-(1→2)-linked D-mannopyranose tetramer. It is, however, very selective in its action since it did not cleave α-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-D-Man. Apparently a D-mannopyranose non-reducing end-unit and two consecutive α-D-mannopyranose residues are required by the enzyme for cleavage of a substrate to take place.

INTRODUCTION

Recently, work has been carried out on the identification and classification of yeasts by the chemical structures of their cell-wall polysaccharides. A D-mannan[1-4], D-galacto-D-mannan[4,5], or a D-mannose-containing heteropolymer[6,7] may be isolated from nearly all species following extraction of the cell-wall with hot aqueous alkali. From all of the yeasts investigated, the polysaccharides contain a main chain consisting of D-mannopyranose residues, which can be linked α-D-(1→3), α-D-(1→6) or can contain alternating β-D-(1→3) and β-D-(1→4) linkages. Two of the three

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main-chain types, the α-(1→3)-D-mannopyranose[6,7] and the alternating β-(1→3) and β-(1→4)-D-mannopyranose[1] are readily characterized by chemical techniques. The former type of main chain can be isolated from a branched polysaccharide by removal of the side chains by one or more Smith degradations[8], the number required depending on the chemical structure of the side chains. The latter type of β-D-linked chain has only been found as a linear polysaccharide[1]. On the other hand, α-(1→6)-D-mannopyranose main chains have been unambiguously demonstrated in only a few D-mannans [3,4] and D-galacto-D-mannans [4,5]. The standard techniques used in structural studies on the polysaccharides give, in most cases, equivocal results. For example, the D-mannan of *Saccharomyces rouxii*, on methylation followed by hydrolysis, gives 2,3,4,6-tetra-*O*-, 3,4,6-tri-*O*-, and 3,4-di-*O*-methyl-D-mannose in an approximately 1:1:1 molar ratio. Coupled with the isolation of an α-(1→2)-linked D-mannopyranose trisaccharide on partial acetolysis, structures of the D-mannan can be proposed varying from I, having an α-D-(1→6)-linked main chain to II having a main chain containing α-D-(1→2) and α-D-(1→6) linkages[9,10]. Gorin and associates have recently investigated the structures of several other D-mannans and D-galacto-D-mannans and have shown, by similar methylation–fragmentation and partial acetolysis techniques, that they contain D-mannopyranose residues which exist in the 2-*O*-, 6-*O*-, and 2,6-di-*O*-substituted forms and as non-reducing end-units, and contain successive α-D-(1→2) linkages[2-5].

A technique has now been provided by G. H. Jones and C. E. Ballou that can lead to the isolation of an α-D-(1→6)-linked mannan chain from D-mannans following enzymolysis[11,12]. *Arthrobacter* GJM-1, a soil bacterium, when grown on a medium containing the D-mannan of baker's yeast (*Saccharomyces cerevisiae*), produces an inducible, exocellular α-D-mannosidase that can preferentially remove individual D-mannose substituents of the side chains of the yeast D-mannan. Similar degradations were carried out on D-mannans from *Candida stellatoidea* and *Kloeckera brevis*. The present publication describes (*A*) the properties of crude exo α-D-mannosidase (which differ somewhat from those of the purified enzyme used by Jones and Ballou), (*B*) the degradation of several D-mannans and D-galacto-D-mannans (previously partly characterized) to fragments containing predominantly α-D-(1→6)-linked D-mannopyranosyl residues, and (*C*) the characterization of β-D-mannose as the hexose anomer formed on enzymolysis of an α-(1→2)-linked, D-mannopyranose tetrasaccharide.

RESULTS AND DISCUSSION

Properties of the crude exo α-D-mannosidase from Arthrobacter GJM-*1*. — Jones and Ballou[11,12] degraded D-mannans with a purified exocellular exo α-D-mannosidase preparation from *Arthrobacter GJM*-1. Since the crude enzyme has the required property of removing side-chain residues from D-mannans, a purification step was not carried out for the present investigations. However, some differences in properties between the two preparations were observed. It was found that the crude α-D-mannosidase degraded the α-D-(1→6)-linked mannan main chains of *Schizo-*

saccharomyces octosporus D-galacto-D-mannan and *S. rouxii* D-mannan, whereas the purified preparation of Jones and Ballou only removes D-mannose side chains[12]. Their enzyme preparation can attack α-D-(1→6)-linked mannose oligosaccharides, but not the main chain of *S. cerevisiae* D-mannan. Initially our degradations of the D-mannans of *S. octosporus* and *S. rouxii* proceeded at rates that were somewhat less than that for the D-mannan of *S. cerevisiae*, as evidenced by the production of D-mannose (Fig. 1). Only limited degradations of each of the two main chains took

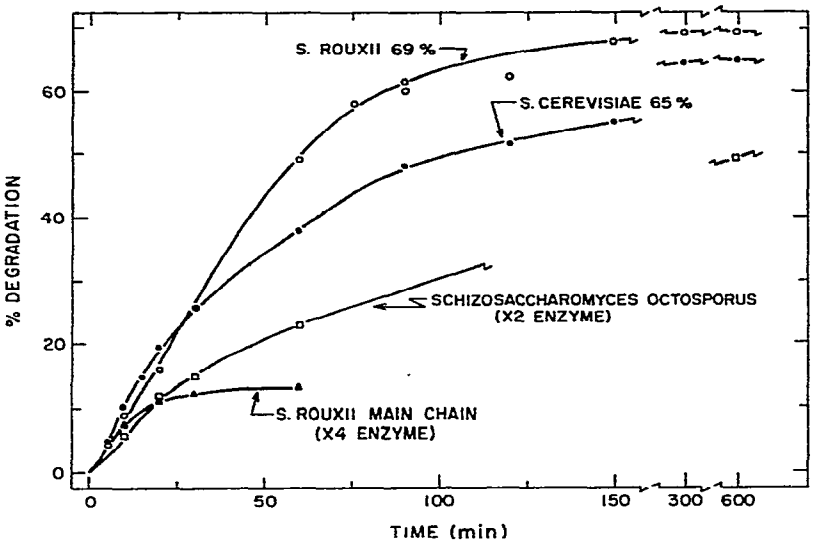


Fig. 1. Degradation of yeast D-mannans with α-D-mannosidase. The relative initial degradation rates were: yeast D-mannan (*S. cerevisiae*), 1.0; *S. rouxii* D-mannan, 0.9; *S. octosporus* main chain, 0.285; *S. rouxii* main chain, 0.18.

place, presumably because the exo enzyme reached resistant units in the chains. Since the main chains were partly vulnerable to attack, a limited degradation time of 10 h was used (activity of crude enzyme in 1% solution, 0.169 units/ml; specific activity, 0.422). This is sufficient to degrade the 7-unit, α-(1→2)-linked, D-mannopyranosyl side chain of *T. bombi* mannan completely. The corresponding α-D-(1→2)-linked octasaccharide isolated following partial acetolysis undergoes complete degradation to D-mannose in 6 h.

The crude preparation contains enzymes other than α-D-mannosidase. Jones and Ballou, when using a purified α-D-mannosidase lacking phosphatase and protease activity[11], noted that the extent to which several D-mannans from *Candida* spp. were hydrolyzed was inversely proportional to their phosphorus content[12]. (*Kloeckera brevis* D-mannan, which is heavily phosphorylated, proved an exception). Disc-electrophoretic examination of our crude enzyme at pH 8.3 showed six major and seven minor protein components. One of these components was an active alkaline phosphatase [a 1% solution of the crude lyophilized enzyme had 3.78 units/ml at pH 7.0 (4.12 units/ml at pH 8.5), specific activity 9.45].

B. *Enzymolysis of* D-*mannans and acid-degraded* D-*galacto*-D-*mannans and* D-*mannans.* — Some of the D-mannans, for which some structural information is available, could be degraded with the crude exo α-D-mannosidase to their α-(1→6)-linked D-mannopyranose main chains. These are from *Endomycopsis fibuliger*[4], *Saccharomyces rouxii*[9,10], *Torulopsis apicola* (Hajsig strain), *Torulopsis bombi*[4], and *Candida parapsilosis*[13]. The end-products were characterized, following their isolation by deionization followed by ethanol precipitation, by the H-1 proton magnetic resonance (p.m.r.) signal at τ 4.57 (*J* 1 Hz). This signal has the same chemical shift as that of H-1 of α-D-(1→6)-linked mannans formed by partial acid hydrolysis of the D-galacto-D-mannan of *Schizosaccharomyces octosporus*[3], and by partial enzymolysis of the D-mannan of *S. cerevisiae*[12]. Addition of sodium borate shifts the signal downfield by approximately 10 Hz because of the formation of a 2,3-substituted borate complex[2]. These properties distinguish the H-1 signal from those of α-D-(1→2)-linked (τ 4.20) and α-D-(1→3)-linked (τ 4.35) polymers, which do not exhibit such a large downfield shift on the addition of borate[2].

The above direct enzymolysis technique could not be used on D-galacto-D-mannans having D-galactosyl side chains and D-mannans containing β-linkages in the side chains. D-Galacto-D-mannans containing D-galactopyranose non-reducing end-units isolated from *Trichosporon fermentans*[5], *Torulopsis magnoliae*, *Torulopsis gropengiesseri*, *Candida lipolytica*, and *Torulopsis lactis-condensi*[4] were resistant to the action of the crude α-D-mannosidase. Mannans from *Saccharomyces lodderi*, *Citeromyces matritensis*, and *Pichia pastoris*[3], which contain β-linkages in the side chains were similarly resistant. The D-mannan of *Trichosporon aculeatum*[2] appears to have a minor proportion of β-D-linked residues, as evidenced[3] by a small signal (13%) at a relatively high field of τ 4.62. It was only partially degraded by the α-D-mannosidase because of the lack of accessibility of the α-D-linked units. In order to render each of these polysaccharides vulnerable to attack by α-D-mannosidase, it was first necessary to remove the D-galactopyranosyl residues or β-D-linked residues. This was accomplished by partial acid hydrolysis with 0.33N sulfuric acid at 100°.

The α-D-galactopyranosyl end-units in *T. fermentans*, *C. lipolytica*, and *T. lactis-condensi* D-galacto-D-mannans were mostly removed by partial hydrolysis, only traces of D-galactose residues remaining in the degraded polysaccharides. β-D-Galactopyranosyl end-units in the D-galacto-D-mannans of *T. gropengiesseri* and *T. magnoliae* were indicated by methylation–fragmentation analyses and the D-galacto-D-mannans appear to be β-D-linked since acid degradation gives polymers having higher specific rotations than the starting materials (Table I). β-Linked D-mannopyranosyl residues should be absent since H-1 signals at higher field than τ 4.53 were not detected in the parent D-galacto-D-mannan. On partial hydrolysis the β-D-galactopyranosyl non-reducing end-units were completely removed from *T. gropengiesseri* and *T. magnoliae* D-galacto-D-mannans, but the D-mannan from the latter appeared to be contaminated with a trace of a D-glucan.

The β-linked D-mannopyranosyl side-chain residues in the D-mannans of *S. lodderi*, *C. matritensis*, and *P. pastoris* were mostly removed, since the acid-

TABLE I

YIELD AND SPECIFIC ROTATIONS OF ACID-DEGRADED, CELL-WALL POLYSACCHARIDES AND THE OVERALL YIELDS OF ENZYME-DEGRADED POLYSACCHARIDES

Polysaccharide[a] *(reference)*	*Acid-degraded* D-*mannan* *Yield* (%)	$[\alpha]_D$, *degrees* (*c*, 0.5%)	*Enzyme-degraded* D-*mannan* *Yield* (%)
D-Galacto-D-mannans			
Candida lipolytica CBS 599 (4)	45	+86	17
Torulopsis gropengiesseri NRRL-Y1445 (4)	22	+74[b]	8
Torulopsis lactis-condensi CBS 52 (4)	29	+77	7
Torulopsis magnoliae CBS 166 (4)	16	+69[c]	3
Trichosporon fermentans PRL 2263 (5)	31	+92	27
Mannans having β-D-linkages			
Citeromyces matritensis CBS 2764 (3)	19	+44	11
Pichia pastoris PRL 63-208 (3)	33	+57	22
Saccharomyces lodderi JPV 193 (3)	47	+69	17
Trichosporon aculeatum IGC 3551 (4)	40	+71	30
α-D-linked mannans			
Candida parapsilosis BMCI (3, 13)			30
Endomycopsis fibuliger NCYC 13 (4)			38
Saccharomyces rouxii PRL 411-64 (9, 10)			22
Torulopsis apicola (Hajsig strain) CBS 2868 (4)			36
Torulopsis bombi 319-67 (4)			11

[a]CBS, Centraalbureau voor Schimmelcultures, Yeast Division, Delft, The Netherlands; PRL (also BMC), Prairie Regional Laboratory, National Research Council, Saskatoon, Saskatchewan, Canada; NCYC, National Collection of Yeast Cultures, Brewing Industry Research Foundation, Nutfield, Surrey, England; JPV, Collection of J. P. van der Walt, Pretoria, South Africa; IGC, Gulbenkian Institute of Science, Oerias, Portugal; NRRL, Northern Regional Research Laboratory, USDA, Peoria, Illinois, USA. [b]Undegraded polysaccharide[4] has $[\alpha]_D$ +46°. [c]Undegraded polysaccharide[4] has $[\alpha]_D$ +42°.

degraded D-mannans had specific rotations close to the value of +88° reported for an α-D-linked mannan[14]. In addition, the H-1 p.m.r. signals of the degraded D-mannans contain greatly decreased proportions of the high-field signals that are characteristic of β-D-mannopyranoside linkages (Fig. 2). The yields of acid-degraded polysaccharides are presented in Table I.

Most of the above acid-degraded polysaccharides could then be degraded by the α-D-mannosidase to polysaccharides giving predominantly H-1 signals at approximately τ 4.57, which were shifted downfield by addition of sodium borate. The lack of complete degradation to pure α-(1→6)-linked D-mannopyranose main chains is probably due to the incomplete removal of α-D-mannosidase-resistant, side-chain residues. The H-1 p.m.r. spectra of the polysaccharide substrates, the degraded polysaccharides formed on partial acid hydrolysis, and the products formed on enzymolysis are presented in Fig. 2.

The isolation of fragments consisting predominantly of α-(1→6)-linked D-mannopyranose units from D-mannans of *E. fibuliger*[4], *P. pastoris*, and *C. matri-*

tensis[3] and the D-galacto-D-mannan of *T. fermentans*[5] is useful, since it means that the predominating structures, previously postulated on the basis of chemical evidence,

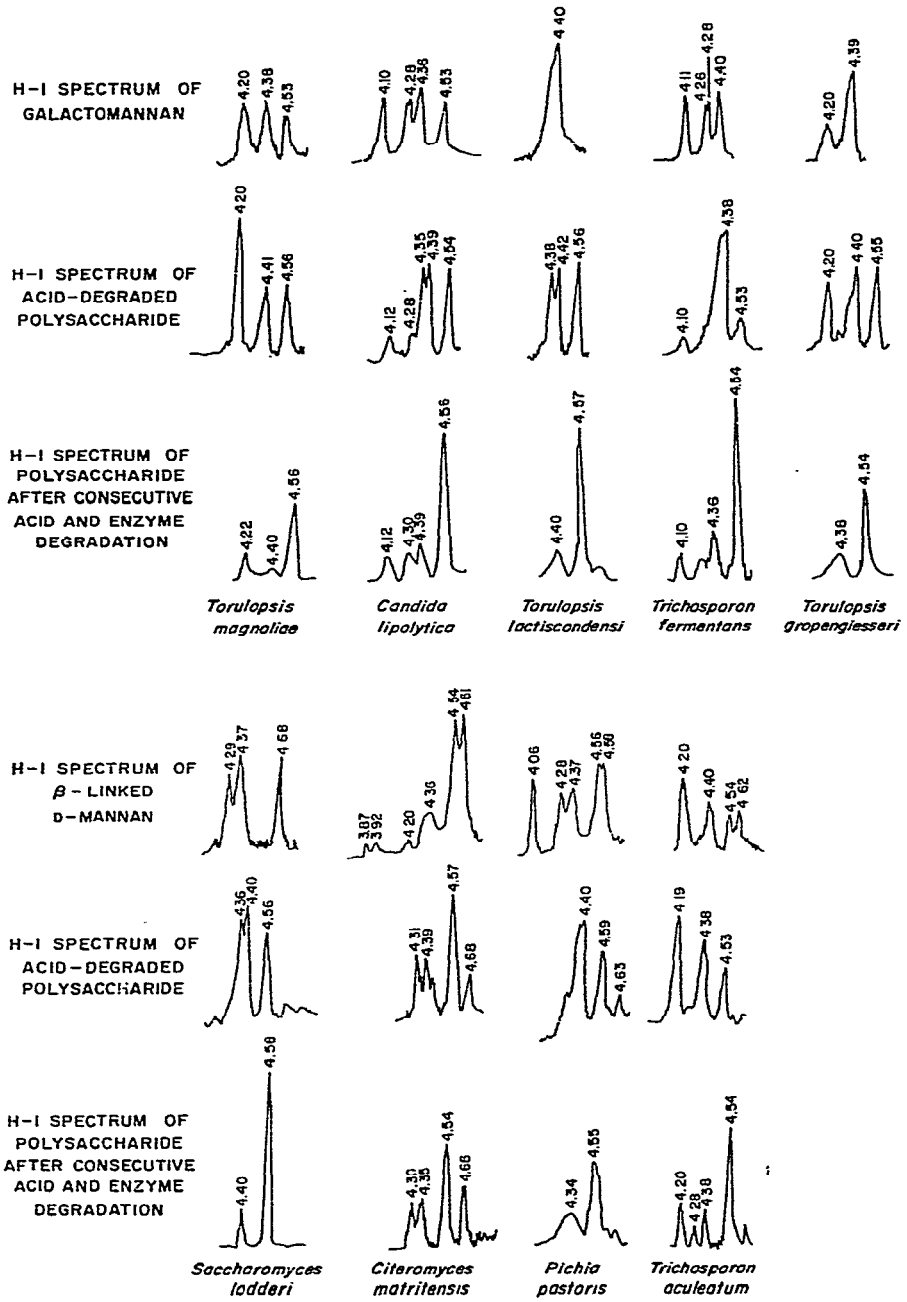


Fig. 2. P.m.r. spectra of D-galacto-D-mannans, β-linked D-mannans and the polysaccharides formed on successive degradations with acid and D-mannosidase.

TABLE II

NATURE OF SIDE CHAINS IN D-MANNOSE-CONTAINING POLYSACCHARIDES HAVING α-(1→6)-D-MANNOPYRANOSE MAIN CHAINS

Source of D-*mannose-containing polysaccharides*	*Largest side-chain substituents linked* α-D-(1→2) *to* α-(1→6)-D-*mannopyranose main chain*	*Polysaccharide contains* 6-O-*substituted* α-D-*mannopyranose residues according to* τ 4.57 *signal*
D-Galacto-D-mannans		
Candida lipolytica[4]	α-D-Gal*p*-(1→2)-D-Man*p*	+
Torulopsis gropengiesseri[4]	β-D-Gal*p*-(1→?)-α-D-Man*p*-(1→2)-D-Man*p*	−
Torulopsis lactis-condensi[4]	α-D-Gal*p*-(1→6)-α-D-Man*p*-(1→2)-D-Man*p*	−
Torulopsis magnoliae[4]	α-(1→2)-linked, 9-unit D-Man*p* side chain terminated by Gal*p* non-reducing end-unit with β-D-linkage	+
Trichosporon fermentans[5]	α-D-Gal*p*-(1→2)-D-Man*p*	trace
D-Mannans having β-D-linkages		
Citeromyces matritensis[3]	β-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-D-Man*p* (linked β-D-(1→2)-to main chain)	?
Pichia pastoris[3]	α-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-D-Man*p*	?
Saccharomyces lodderi[3]	—	
Trichosporon aculeatum[2]	α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-D-Man*p*	+
α-Linked D-mannans		
Candida parapsilosis[3,13]	Not known	+
Endomycopsis fibuliger[3]	α-D-Man*p*-(1→3)-D-Man*p* and-D-Man*p*	−
Saccharomyces rouxii[9,10]	α-D-Man*p*-(1→2)-D-Man*p*	−
Torulopsis apicola[4]	α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-D-Man*p*	+
Torulopsis bombi[4]	α-(1→2)-Linked, 7-unit D-Man*p* side chain.	+

are now confirmed. It should be emphasized, however, that on the basis of present data, the possibility of 6-*O*-substituted branch-points in the side chains is not eliminated and our postulated structures may be oversimplified.

The isolation of main-chain fragments from D-mannose-containing polysaccharides is useful when considered in the light of partial acetolysis data previously published (references presented in Table II). The nature of the oligosaccharides isolated from each polysaccharide show the structures of the side chains that are attached by α-D-(1→2)-links to the α-D-(1→6)-linked mannopyranose main chain (Table II). Some of these polysaccharides contain very long side chains, up to 9 units in length. *Since many yeast* D-*mannans and* D-*galacto*-D-*mannans contain* α-(1→6)-D-*mannopyranose main chains, it is evident that the wide diversity of H-1 p.m.r. spectra from polysaccharides having this backbone is caused by differences in side chain structure.*

Two D-mannans from *Hansenula subpelliculosa* and *Candida catenulata* were only partly degraded by the α-D-mannosidase, with only small increases in the H-1 p.m.r. signal at τ 4.57, characteristic of the α-(1→6)-linked D-mannopyranose chain. In cases where a polysaccharide gives an H-1 p.m.r. signal at τ 4.57, this is taken as evidence of unsubstituted 6-*O*-linked, α-D-mannopyranose main-chain residues. This is of particular interest since the specific rotations of the undegraded D-mannans, +71° and +110°, respectively[4], indicate a preponderance of α-D-mannosidic linkages and few, if any, enzyme-resistant β-D-linkages. It therefore appears that not all α-linked D-mannopyranose residues can be cleaved with the exo α-D-mannosidase.

C. *Mechanism of enzymolysis of* α-(1→2)-*linked* D-*mannopyranose tetramer.* — Oligosaccharide fragments obtained by partial acetolysis of the above polysaccharides, and which contain either β-D-mannopyranose or D-galactopyranosyl residues, were not attacked by the crude α-D-mannosidase. This contrasts with the breakdown of oligosaccharides containing only α-(1→2), or mixed α-(1→2) and α-(1→3) D-mannopyranose residues (refs. 11, 12, and Table III). The resistance of 2-*O*-linked tetra-

TABLE III

SPECIFICITY OF ACTION OF CRUDE EXO α-D-MANNOSIDASE OF OLIGOSACCHARIDES CONTAINING D-MANNOSE RESIDUES

Oligosaccharides degraded by crude α-D-*mannosidase*

1 α-D-Man*p*-(1→2)-D-Man[9]
2 α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-D-Man[3]
3 α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-D-Man[4]
4 α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-D-Man[4]
5 α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-D-Man[4]
6 α-(1→6)-Linked D-mannopyranose main chains from *S. octosporus*[3] and *S. rouxii*.

Oligosaccharides resistant to the crude α-D-*mannosidase*

7 α-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-D-Man[3]
8 α-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-D-Man[3]
9 β-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-D-Man[3]
10 β-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-D-Man[3]
11 α-D-Gal*p*-(1→2)-D-Man[3]
12 α-D-Gal*p*-(1→2)-α-D-Man*p*-(1→2)-D-Man[5]
13 α-D-Man*p*-(1→6)-D-G[19]

saccharide **7** and pentasaccharide **8**, which contain α- and β-linkages (Table III) to cleavage by an exo-enzyme at the non-reducing, α-D-linked end demonstrates a high degree of specificity for the enzyme. Since the bacterial enzyme attacks *p*-nitrophenyl α-D-mannopyranoside slowly and methyl α-D-mannopyranoside not at all[11] it appears that the enzyme requires an α-D-mannopyranose non-reducing end and two consecutive α-D-mannopyranosyl residues. The enzyme resembles an exo-amylase from microorganisms that can cleave amylose, but not methyl α-D-glucopyranoside[14,15].

Jones and Ballou[12] have shown that degradation of branched-chain oligosaccharides (from the D-mannan of *S. cerevisiae*) with the exo α-D-mannosidase proceeds in a stepwise fashion starting from the non-reducing ends. Further studies of the reaction mechanism have now been made by using the p.m.r. technique of Eveleigh and Perlin[16]. An α-(1→2)-linked, D-mannopyranose tetrasaccharide was used as substrate rather than a polysaccharide since it gave an H-1 p.m.r. spectrum that is better defined. The α-D-mannosidase had been concentrated for the p.m.r. spectrum by precipitation with ammonium sulfate. Enzymolysis gave β-D-mannose (showing an H-1 signal at τ 4.65, *J* 1 Hz) and a high proportion of trisaccharide (paper chromatogram) in the early stages (4 min). After β-D-mannose had been formed in 14% yield, α-D-mannose (τ 4.40, *J* 1 Hz, H-1), resulting from mutarotation, could be detected (Fig. 3). The occurrence of anomeric inversion at C-1 is similar

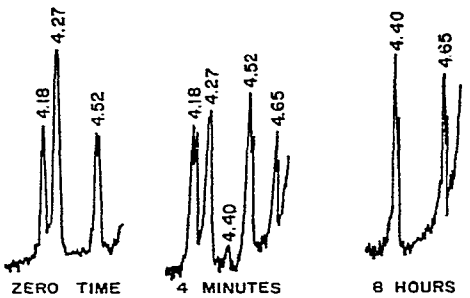
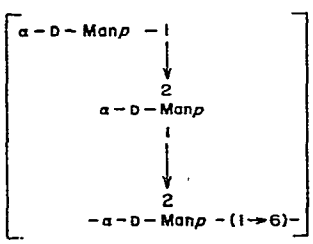


Fig. 3. Course of D-mannosidase enzymolysis of α-(1→2)-linked D-mannopyranose tetrasaccharide, as followed by p.m.r. spectroscopy of the H-1 region.

to the findings for polysaccharases by Eveleigh and Perlin[16]. They stated that, in general, D-glucanases of the exo type, which attack from the non-reducing chain-end,



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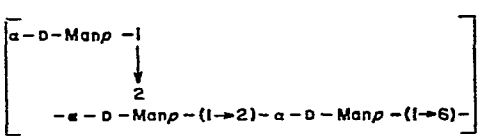

II

cause inversion of anomeric configuration, whereas D-glucosidases and D-glucanases of the endo type, which attack the chain randomly, give products in which the anomeric configuration of the substrate is retained. One apparent exception to these generalizations is an endo-dextranase, which gives products in which the configuration of the product differs from the substrate[17]. Although the enzyme of Jones and Ballou was originally called an α-D-mannosidase[11], it does not degrade glycosides of D-mannopyranose, but can degrade α-(1→6)-linked D-mannopyranose main chains (see above). It therefore has some of the properties of an exo α-D-mannanase and the crude enzyme may possibly be named an α-1→(2, 3 or 6)-D-mannanase preparation (according to a private communication from E.T. Reese).

EXPERIMENTAL

Measurement of p.m.r. spectra. — The H-1 spectra of polysaccharides were obtained from D_2O solutions at 70° by using a Varian 100-MHz spectrometer and a sweep width of 1000 Hz. The concentration of polysaccharide used was 10%. Tetramethylsilane was used as an external standard and was contained in a capillary tube mounted coaxially inside the p.m.r. tube. Chemical shifts are expressed on the τ scale.

Partial acid hydrolysis of D-mannose-containing polysaccharides. — In a typical experiment the polysaccharide (0.60 g) was treated[5,18] for 18 h in 0.33N sulfuric acid (6 ml) at 100°. The degraded polysaccharide was precipitated by addition of ethanol (50 ml). The precipitate was centrifuged and washed successively once with ethanol and twice with acetone. The yields, specific rotations, and sugar compositions of the products are recorded in Table I. The sugar compositions of acid-degraded polysaccharides and enzyme-degraded polysaccharides were determined by hydrolysis with N H_2SO_4 for 18 h at 100° followed by paper chromatography with butyl alcohol–ethanol–water (40:11:19 v/v) as solvent and *p*-anisidine hydrochloride as spray reagent.

Preparation of exo α-D-mannosidase of Arthrobacter GJM-*1.* — The enzyme was obtained by growth of the microorganism on a medium of baker's yeast D-mannan according to the method of Jones and Ballou[11]. For enzymolysis of D-mannans, a crude preparation was used consisting of exocellular medium that had been lyophilized following dialysis against phosphate buffer. A 20% solution of enzyme in D_2O was examined by p.m.r. spectroscopy and was shown to be carbohydrate-free. Some of the enzyme was purified by fractional precipitation by ammonium sulfate up to a concentration of 55%, and was utilized in the enzymolysis of an α-(1→2)-linked D-mannose tetrasaccharide to β-D-mannose (see below).

Enzyme assays. — A minor modification of the α-D-mannosidase assay procedure of Jones and Ballou was used[11]. The assay mixture consisted of yeast D-mannan (400 μg), calcium chloride (0.1 μM) in 0.1M potassium phosphate buffer (0.50 ml, pH 6.8) and enzyme, which was diluted to 1.0 ml with water. After incubation for 10 min at 30°, alkaline Somogyi–Nelson[20] copper reagent (0.5 ml) was added.

The reaction mixture was heated for 30 min at 100° and reducing groups were estimated by using the arsenomolybdate reagent[20]. An approximately linear relationship between enzyme concentration and product was found up to 45% substrate degradation (Fig. 1). Repeated assays showed that the initial reaction rate was slightly lower than the rate attained after 0.5% degradation of the substrate. A unit (U) of activity is defined as that amount of enzyme which causes the release of 1 μmole of D-mannose (or its equivalent) per min under these conditions. One U equals 85.8 units of Jones and Ballou[11]. Specific activity is defined as unit of activity of enzyme per mg of protein[21]. Alkaline phosphatase was assayed by using *p*-nitrophenyl phosphate as a substrate[22]. A unit is defined as the release of 1 μmole of *p*-nitrophenol/min; the molar absorbancy index for *p*-nitrophenol in M Tris buffer (pH 8.0) equals 1.62×10^4.

Enzymolysis of polysaccharides. — The substrate (50 mg) was dissolved in water (2 ml) and treated with crude enzyme having an activity (in 1% solution) of 0.169 U/ml; specific activity 0.422. After the required time the enzyme was deactivated for 10 min at 100° and salts were then removed by adding mixed resins consisting of Amberlite IR-120 (H^+) form and Dowex-1 (hydrogen carbonate form). The filtered solution was then evaporated to approximately 0.3 ml and could be applied directly to the paper chromatograms. The polysaccharide residue was precipitated by addition of excess ethanol and the residue, after centrifugation, was washed with ethanol and then with acetone. The yields of products, based on the original polysaccharides, are presented in Table I. The specific rotations of the enzyme-degraded polysaccharides in H_2O are $+80°$ ($\pm 15°$).

Enzymolysis of α-linked D-*mannopyranose tetrasaccharide.* — Enzyme (4.9 U) that had been precipitated by ammonium sulfate (30–55%) was added to a solution of the tetramer (30 mg) in D_2O (0.7 ml) at 30° and the enzymolysis was followed by the change of the H-1 signals in the p.m.r. spectrum. Initially β-D-mannose (H-1, τ 4.65) was formed, but anomerization to α-D-mannose (H-1, τ 4.40) was detected after 4 min. The H-1 signals of the two anomers were distinguishable from those of the starting material (τ 4.19, 4.25, and 4.52), and other degradation products having similar chemical shifts, namely 2-*O*-α-D-mannopyranosyl-D-mannose and a trisaccharide having α-(1→2)-linked D-mannopyranose residues (Fig. 3). The trisaccharide and D-mannose were detected on paper chromatograms [solvent: 2:1:1 (v/v) butyl alcohol–ethanol–water; spray: ammoniacal silver nitrate] after 6 min.

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