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Expression of α-1,3 linkage-containing oligomannosyl residues in a cell-wall mannan of *Candida tropicalis* grown in yeast extract–Sabouraud liquid medium under acidic conditions

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Abstract

We investigated the cell-wall mannan obtained from *Candida tropicalis* IFO 1647 strain cells grown in yeast extract–Sabouraud medium at pH 3.0 by two-dimensional homonuclear Hartmann–Hahn spectroscopy. The results indicate that the phosphate group and the side chains containing a β-1,2-linked mannopyranose unit decreased compared to those of mannan from cells grown under conventional conditions (pH 5.9) with concomitant expression of α-1,3 linkage-containing oligomannosyl side chains. The results of acetolysis of these mannans indicated that the presence of α-1,3-linked mannopyranose unit existed in side chains corresponding to pentaose and hexaose, Manpα1–3Manpα1–2Manpα1–2Manpα1–2Man, and Manpα1–2Manpα1–3Manpα1–2Manpα1–2Manpα1–2Man, in the mannan from cells grown at pH 3.0.

Key words: Yeast cell-wall mannan; Chemical structure; Nuclear magnetic resonance; α-1,3 Linkage; β-1,2 Linkage; *Candida tropicalis*

1. Introduction

We have reported the structural analysis of cell wall mannans of three pathogenic *Candida* species, *C. albicans* [1], *C. stellatoidea* [2], and *C. tropicalis* [3]. These mannans were composed of a long core (backbone) consisting of a α-1,6-linked mannopyranose unit and a large number of oligomannosyl side chains. The long side chains containing both β-1,2 and α-1,2 linkages were found to function as the serotype A-specific epitope of *C. albicans* species [4]. We have also found that *C. albicans* serotype A strains were able to grow in yeast extract-Sabouraud liquid medium at pH 2.0 with a flocculation in grape-like clusters, whereas a *C. albicans* serotype B strain could not, and that the mannans of the former serotype A strains grown in this medium contained neither phosphate nor β-1,2-linked mannopyranose unit [5].

Recently, we reported the structures of *C. tropicalis* IFO 0199 and IFO 1647 strain mannans [3], revealing that the mannans obtained from the cells grown in the conventional medium (pH 5.9) were found to entirely lack the α-1,3-linked mannopyranose unit, a striking difference from those of the other *Candida* species. Thereafter, it was incidentally found that the cells of *C. tropicalis* strains were able to grow in yeast extract–Sabouraud liquid medium at pH 3.0 exhibiting a remarkable floc-forming ability, whereas any growth was not observed at pH 2.0 (unpublished data). We attempted the analysis of the structural difference between mannans obtained from the *C. tropicalis* IFO 1647 strain cells grown under conventional (pH 5.9) and acidic (pH 3.0) conditions, because findings obtained from this study seem to be useful for the elucidation of the biosynthetic process of *Candida* mannans.

2. Materials and methods

2.1. Strain and cultivation

The *C. tropicalis* IFO 1647 strain (1647-strain) was obtained from the Institute for Fermentation Osaka (IFO), Japan. This strain was cultivated on yeast extract-Sabouraud medium of two different pH's, 5.9 ± 0.1 and 3.0 ± 0.1. The medium of pH 5.9 was prepared by autoclaving of a 200-ml batch of 0.5% (w/v) yeast extract, 1% (w/v) peptone, and 2% (w/v) D-glucose contained in a 500-ml flasks for 20 min at 121°C. Adjustment of pH to 3.0 was readily achieved by the addition of 6 N HCl to the pH 5.9 medium. Then the cells of 1647-strain precultivated in the pH 5.9 medium at 27°C, each 10^5 cells in 100 μl of saline, were separately inoculated to either medium of different pH, and cultivation was conducted on a reciprocal shaker for 72 h at 27°C.

2.2. Preparation of mannans

This was performed as described previously [6]. The mannan fractions obtained from the cells grown in two media of different pH, 5.9

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and 3.0, were designated as Frs. T6 and T3, respectively. The yields of Frs. T6 and T3 were 3.8% and 3.1%, respectively, on weight basis of the acetone-dried whole cells.

2.3. Two-dimensional homonuclear Hartmann–Hahn (2D-HOHAHA) analysis

2D-HOHAHA spectra were recorded on a Jeol JNM-GSX 400 spectrometer (400 MHz) in accordance with the description of Shibata et al. [7].

2.4. Pretreatment of Frs. T6 and T3 with diluted acid and alkali

This was done as described by Shibata et al. [8]. The yields of the released oligosaccharides from Frs. T6 and T3 were 3.3% (w/w) and 0.9 % (w/w), respectively. The modified Frs. T6 and T3 were designated as Frs. T6-ab and T3-ab, respectively.

2.5. Acetolysis of Frs. T6-ab and T3-ab

This was done as described previously [6] by a modification of the method of Kocourek and Ballou [9]. The resultant oligosaccharides were fractionated on a column (2.5 × 100 cm) of Bio-Gel P-2 (400 mesh, Bio-Rad, CA) and eluted with water (0.25 ml/min). The carbohydrate content of eluates was determined by the phenol/H_2SO_4 method [10]. Each oligosaccharide corresponding to the peaks in the elution profile was rechromatographed on the same column to remove contaminated lower and/or higher oligosaccharides.

2.6. Another method

Phosphate content of mannans was determined by the method of Ames and Dubin [11] using KH_2PO_4 as a standard.

3. Results

The structural difference between Frs. T6 and T3 was investigated by adopting 2D-HOHAHA spectroscopy. Fig. 1A and B show the spectra of Frs. T6 and T3. Assignment of the H1–H2 cross-peaks was done as described by Shibata et al. [12], and summarized in Table 1. These data indicate that the Fr. T3 lacks 1-*O*-α-phosphorylated glycose unit corresponding to H1 signals, 5.563 and 5.542 ppm (cross-peak 1), which can be seen in the spectrum of Fr. T6, and concomitantly decreases one of the serotype A-specific epitopes of the *C. albicans* species, Man*pβ*1–2Man*pβ*1–2Man*pα*1–2Man*p*, corresponding to the cross-peaks 5b, 13, and 14. The fact that the phosphate was not detected in Fr. T3 supported the result of the 2D-HOHAHA analysis. In contrast, appearance of the different cross-peaks, 2–8, in the spectrum of Fr. T3 (Fig. 1B) suggests the expression of several side chains containing an α-1,3-linked mannopyranose unit and another serotype A-specific epitope of *C. albicans* species, Man*pβ*1–2Man*pα*1–2Man*p*, corresponding to the cross-peaks 5a and 8a, which were not detected in the previous structural study of Fr. T6 [3].

The mannans were subjected to acetolysis in order to detect the side chains containing an α-1,3-linked mannopyranose unit in Fr. T3. Before acetolysis, Frs. T6 and T3 were treated with dilute acid and alkali to eliminate the oligomannosyl units, which were linked to the phosphate group and peptidic moiety of these mannans. Fig. 2A and B show the elution profiles of the resultant products from the acid and alkali-treated mannans, Frs. T6-ab and T3-ab, by acetolysis. In Fig. 2A, the oligosaccharides obtained from Fr. T6-ab, from pentaose (M_5) to biose (M_2), were identified to be a homologous series of α-1,2-linked ones using the ^{1}H-NMR analysis by correlation with data in the literature [3,6] (data not shown). On the other hand, the appearance of hexaose (M_6) in acetolysates obtained from Fr. T3-ab was evident (Fig. 2B). The results of 2D-HOHAHA of the low molecular oligosaccharides, M_4, M_3, and M_2, obtained from Fr. T3-ab indicate that these were composed of α-1,2-linked mannopyranose unit (Fig. 3A, data for M_3 and M_2 are omitted). However, the presence of cross-peaks 6 and 7 in the spectra of the high molecular oligosaccharides, M_5 and M_6, indicates that the molecules, which have each one terminal α-1,3-linked mannopyranose unit, exist together with the oligosaccharides consisting solely of α-1,2-linked ones in these compounds (Fig. 3B and C). In addition, the presence of the cross-peak 2 in Fig. 3C indicates that the M_6 contained an internal α-1,3 linkage-containing molecule, Man*pα*1–2Man*pα*1–3Man*pα*1–2Man*pα*1–2Man*pα*1–2Man.

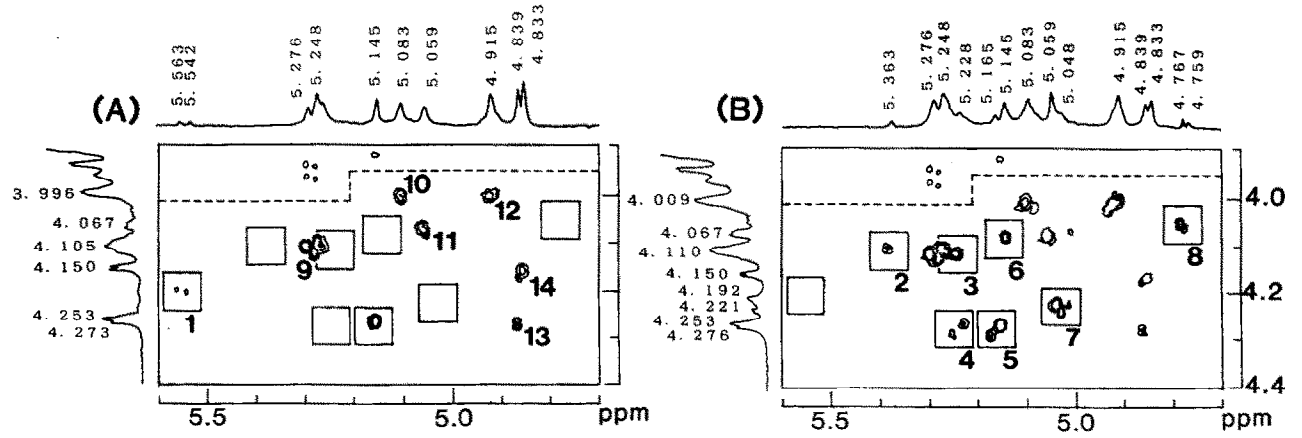

Fig. 1. Partial 2D-HOHAHA spectra of mannans, Frs. T6 (A) and T3 (B). Each mannan was dissolved in D_2O in 1% (w/v), and the determination was conducted at 45°C using acetone (2.217 ppm) as an internal standard. The presence of intraresidues H1–H2 cross-peaks for each mannose unit is shown below the dashed line.

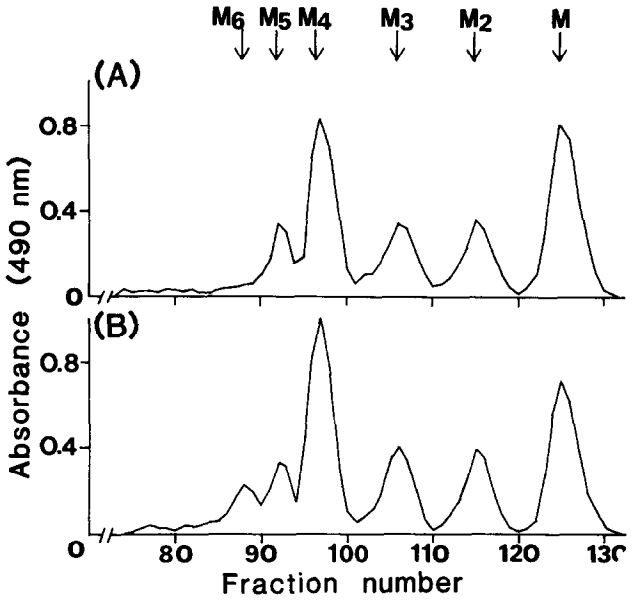

Fig. 2. Elution profiles of the resultant oligosaccharides obtained from Frs. T6-ab (A) and T3-ab (B) by acetolysis. M_6, M_5, M_4, M_3, M_2, and M indicate the manno-oligosaccharides, hexaose, pentaose, tetraose, triose, and biose, and mannose, respectively.

It should be stressed that the structural change of mannan observed in this study was reversible in the range of pH, from 5.9 to 3.0, and that a similar change was observed on the mannan of another *C. tropicalis* strain, IFO 0199, grown under the same conditions (data not shown).

4. Discussion

Recently, it was reported that the β-1,2-linkage-containing oligomannosyl side chains in *Candida* mannans correspond to the adhesion sites to mammalian cells in the host–parasite interaction [13,14]. On the other hand, Stratford [15] stated that one of the receptors for yeast flocculation recognized the α-linked oligomannosyl side chains in the cell-wall mannan. In this study, we observed the concomitant increase of densitiy of α-linked oligomannosyl side chains in Fr. T3 and the floc-forming ability of parent cells (data not shown). This finding seems to substantiate his interpretation [15]. Elucidation of the flocculation mechanism is of interest in relation to that of adherence of *Candida* cells to other cells including those of mammals.

In yeasts, the enzymes responsible for cell wall mannan biosynthesis are localized in internal membranes, the endoplasmic reticulum and Golgi apparatus [16]. In the present study, suppression of the transfer of mannosylphosphate and the β-1,2-linked mannopyranose unit during biosynthesis of the mannan of 1647-strain was observed in the low pH environment of parent cells. Additionally, the transfer of not only the α-1,3-linked mannopyranose unit but also several additional mannopyranose units were observed under the same conditions. The result of acetolysis of Fr. T3-ab indicates that the induction of the activities of α-1,3 and α-1,2 mannosyltransferase, recognizing the tetraosyl and the pentaosyl side chains corresponding to Manpα1–2Manpα1–2Manpα1–2Man and Manpα1–3Manpα1–

Table 1
Assignment of chemical shifts of H1 and H2 signals (cross-peaks of 2D-HOHAHA) of mannopyranose residues

H1–H2 cross-peak	Chemical shift (ppm)[a]		Sugar residue[b]	H1–H2 cross-peak	Chemical shift (ppm)		Sugar residue
	H1	H2			H1	H2	
1[a]	5.563 5.542	– –	β1-2Mα1-H_2PO_3	8(a)	4.767	4.043	Mβ1-2(Mα1-2)
				8(b)*	4.759	4.031	Mβ1-2(Mα1-3)
2	5.363	4.542	α1-2Mα1-3				
				9	5.248 5.276	4.105 4.110	α1-2Mα1-2
3*	5.228	4.110	(α1-3M)α1-2Mα1-2 |6 α1				
				10*	5.083	3.996 4.009	α1-6Mα1-6 |2 α1
4(a)*	5.240	4.276	β1-2Mα1-3				
4(b)*	5.228	4.253	(β1-2M)β1-2Mα1-3	11	5.059	4.067	Mα1-2
5(a)	5.165	4.276	β1-2Mα1-2	12(a)	4.905	3.996	α1-6Mα1-6
5(b)	5.145	4.253	(β1-2M)β1-2Mα1-2	12(b)*	4.905 4.924	3.996 4.009	Mα1-6
6	5.145	4.067	Mα1-3				
				13	4.839	4.273	Mβ1-2(Mβ1-2)
7	5.048	4.192 4.221	α1-3Mα1-2				
				14	4.833	4.150	β1-2Mβ1-2

[a] Chemical shift was indicated based on acetone (2.217 ppm) as an internal standard.
[b] M denotes a D-mannopyranose residue.
* The assignment of these cross-peaks is an estimation from the result of the analysis of *Saccharomyces kluyveri* mannan by Shibata et al. [12].

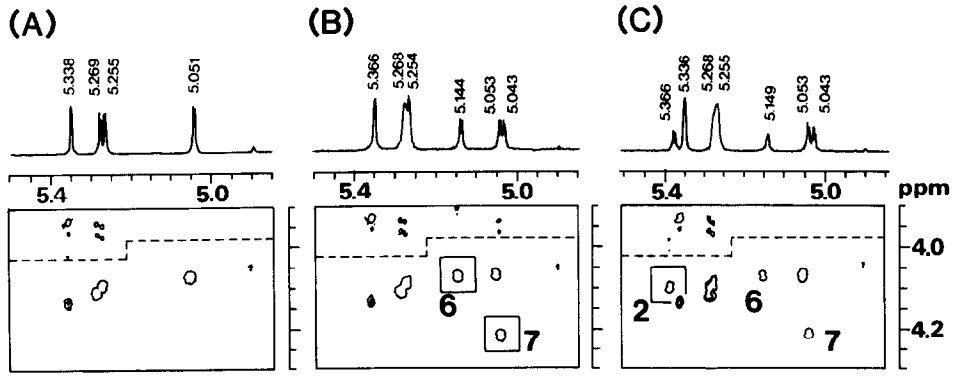

Fig. 3. Partial 2D-HOHAHA spectra of manno-oligosaccharides, M_4 (A), M_5 (B), and M_6 (C), obtained from Fr. T3 by acetolysis. These were recorded under the same conditions as in Fig. 1.

2Man*p*α1–2Man*p*α1–2Man, respectively, took place in the low pH environment of parent cells. The result of 2D-HOHAHA analysis of Fr. T3 indicates the possibility that the activities of other downstream enzymes, β-1,2 and α-1,6 mannosyltransferases, recognizing α-1,3 linkage-containing oligomannosyl side chains were induced. Therefore, it was demonstrated that the 1647-strain has some latent genes corresponding to the inactive enzymes in a conventional environment (pH 5.9), and these enzymes undergo activation to manifest the functions, when the parent cells were grown in a low pH environment.

As the influence of the low pH environment, the following possibilities can be considered; a direct effect to several secretory organella, particularly the endoplasmic reticulum and/or Golgi-apparatus, or an indirect consequence of some other defect, such as the induction of activity of additional factors in relation to expression and/or suppression of correlating genes, and so on. However, it may be presumed that two membrane-bound enzymes, β-1,2 and α-1,3 mannosyltransferases compete for the acquisition of GDP-mannose as a mannose donor and α-1,2-linked mannosyl side chain as an acceptor. Therefore, it seems that these mannosyltransfereses are important as the key enzymes for the downstream in biosynthesis of *Candida* mannan, the yeast flocculation, and the adherence to the other cells. In view of these points, we started a biosynthetic study of *Candida* mannans in the hope to elucidate the mechanisms of these objectives.

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