Isolation of Mannooligosaccharides Corresponding to Antigenic Determinants of Pathogenic Yeast Candida catenulata Cell Wall Mannan

Hidemitsu Kobayashi1, Susumu Kawakami1, Yukiko Ogawa1, Nobuyuki Shibata2, Shigeo Suzuki3
1Laboratory of Microbiology, Faculty of Pharmaceutical Science, Nagasaki International University, Sasebo, Nagasaki, Japan
2Department of Infection and Host Defense, Tohoku Pharmaceutical University, Sendai, Miyagi, Japan
3Sendai Research Institute for Mycology, Sendai, Miyagi, Japan
Email: h-kobaya@niu.ac.jp
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ABSTRACT
To investigate the chemical structure of cell wall mannan of pathogenic yeast, Candida catenulata IFO 0745 strain, which possess the epitopes of antigenic factors 1, 9, and 34 to genus Candida, we previously performed the two-dimensional nuclear magnetic resonance (NMR) analysis of this mannan, Fr. C, without the need for harsh procedures. In this study, three oligosaccharides, biose, triose, and tetraose, and mannose were isolated from Fr. C by acetolysis. The results of NMR analysis indicate that the chemical structures of these oligosaccharides were identified to Manα1-2Man, Manα1-2Manα1-2Man, and Manα1-3Manα1-2Manα1-2Man. The most of resultant mannose seems to be originated from the α-1,6-linked mannan backbone which is recognized by antisera to factor 9. The inhibition assay of slide agglutination reaction between Fr. C and antigenic antibodies using three oligosaccharides indicate that the Manα1-2Manα1-2Man and Manα1-3Manα1-2Manα1-2Man possess domains corresponding to immunodominants of antigenic factors 1 and 34, respectively.

Keywords: Cell Wall Mannan; Antigenic Factor; Candida catenulata; Acetolysis; Oligomannosidic Epitope

1. Introduction
Ten rabbit antibodies to antigenic factors of genus Candida (abbreviated as FAbs) were developed to identify clinical isolates from the patients with candidiasis by Fukazawa et al. [1] and Tsuchiya et al. [2,3]. We have reported the structure of cell wall mannans of genus Candida, for examples, C. albicans [4,5], C. tropicalis [6], C. guilliermondii [7], C. glabrata [8], and C. lusitaniae [9]. The determinants of antigenic factors 1, 9, and 34 to genus Candida were linear α-1,2-linked oligomannosyl side chains [10], linear backbone consisting of α-1,6 linkage [11,12], and linear oligomannosyl side chains containing a non-reducing terminal α-1,3 linkage [12], respectively. On the other hand, the antigenic determinants of factors 5 and 6 correspond to two kinds of β-1,2 linkage-containing side chains, a homologous series of β-1,2-linked oligomannosyl side chains [13], side chains composed of β-1,2 and α-1,2 linkages [14], respectively.

In carbohydrate chemistry, acetolysis is the one of the important procedures for the selective cleavage of glycol-
was obtained from the Biological Resource Center, National Institute of Technology and Evaluation, Japan. This strain was cultivated in the yeast extract-Sabouraud’s liquid medium [0.5% (w/v) yeast extract, 1% (w/v) peptone, and 2% (w/v) glucose] at 27°C for 72 hr on a reciprocal shaker.

2.2. Preparation of Mannans

Mannan were extracted with hot-water and precipitated with Fehling solution [4]. The purified mannan obtained from the cells of the *C. catenulata* strain was designated Fr. C. The yields of Fr. C was 8.0% of the dry cell weight.

2.3. Acetolysis of Fr. C

Acetolysis under conventional conditions was carried out as described previously [20] by modifying the method of Kocourek and Ballou [15]. Namely, mannan, 150 mg, was dissolved in 3 ml of anhydrous formamide in 300-ml glass-stoppered round-bottomed flask, and the solution was added a 1:1 (v/v) mixture of (CH₃CO)₂O and anhydrous pyridine, 100 ml. The clear solution was kept at 40°C for 24 hr. The resultant solution was then evaporated in vacuo to dryness. The residue was dissolved in 2 ml of water, applied to a column of Bio-Gel P-2 (-400 mesh), and eluted by the peak. Aliquots (10 μl) of eluates were assayed for carbohydrate content by the phenol-H₂SO₄ method [21]. Eluates corresponding to each peak were combined and lyophilized after concentration in vacuo.

2.4. Calculation of Average Length of Side Chains and the Branching Frequency Value of Fr. C

The average length of side chains (X) and the branching frequency value (Y) of Fr. C were calculated by using the following formula in accordance with previous descriptions [6]:

\[ X = \frac{(A \times 1) + (B \times 2) + (C \times 3) + (D \times 4)}{(A + B + C + D)} \]

and

\[ Y = \frac{(B + C + D) \times 100}{(A + B + C + D)} \]

respectively, where A through D represent the molar proportions of mannose, biose, triose, and tetraose in the gel-filtration profile of the acetolysis products, and the numbers 1 through 4 indicate the degrees of polymerization of the mannose (M₁) and the three oligosaccharides, M₂ through M₄, respectively.

2.5. ¹H-Nuclear Magnetic Resonance (¹H-NMR) Spectroscopy

The NMR spectra conducted on a JEOL JNM-GSX 400 spectrometer at 400 MHz. It was recorded using a 0.5% (w/v) solution of each oligosaccharide in 0.7 ml of D₂O at 45°C. Acetone (2.217 ppm) was used as an internal standard.

2.6. Inhibition Test of Slide Agglutination Assay Using Oligosaccharides

The inhibition assay of slide agglutination of *C. catenulata* cells with factor antibodies (FAbs) was conducted as previously described [13]. FAbs 1, 9, and 34 were prepared by Fukazawa et al. [1]. The inhibitor oligosaccharides, M₂, M₃ and M₄, were obtained from Fr. C by acetolysis.

3. Results and Discussion

The oligosaccharides mixture obtained from Fr. C by acetolysis were fractionated with water by gel-chromatography of Bio-Gel P-2 (Figure 1). The large amounts of oligosaccharides, tetraose (M₄) and triose (M₃), and the small amounts of oligosaccharides, biose (M₂) and mannose (M₁), were eluted. No product eluted at the position of void-volume (Vo) indicates that all α-1,6 linkages in Fr. C were completely cleaved by the acetolysis. The chemical structures of resultant oligosaccharides were analyzed by ¹H-NMR spectroscopy. The H-1 region signals of these oligosaccharides were shown in Figure 2. All spectra were identical to those of M₂, M₃, and M₄, which were previously isolated from the cell wall mannan of *Saccharomyces cerevisiae* wild-type [22] and *Candida glabrata* [8]. The structure of M₂ and M₃ were
Figure 1. Elution profile of oligosaccharides obtained from *C. catenulata* mannan, Fr. C, by conventional acetolysis. $V_o$ refers void-volume region. $M_4$, $M_3$, $M_2$ and $M_1$ indicate the eluted positions of standard monooligosaccharides, tetraose, triose, and biose, and mannose, respectively.

Figure 2. $^1$H-NMR spectra of oligosaccharides obtained from *C. catenulata* mannan, Fr. C, by conventional acetolysis. Symbols are the same as in Figure 1.

identified to Manα1-2Man and Manα1-2Manα1-2Man, respectively. The H-1 signal at 5.144 ppm in the spectrum of $M_4$ indicates the presence of non-reducing terminal α-1,3-linked mannose residue linked to α-1,2-linked oligomannosyl unit. Therefore, the structure of $M_4$ was identified to Manα1-3Manα1-2Manα1-2Manα1-2Man. The chemical structure of all oligosaccharides and the assignment result of chemical shifts of all mannose residues based on the results of previous reports [8,23] were shown in Table 1.

As shown in Table 2, to identify the antigenic determinants in *C. catenulata* mannan corresponding to antigenic factor, we performed an inhibition assay of agglutination between *C. catenulata* cells and factor antibodies, FAb 1, 9, and 34, with three inhibitor oligosaccharides, $M_1$, $M_2$, and $M_3$ (mannose) obtained from Fr. C by acetolysis. The fact that the antigen determinant of factor 9 could not be found in this experiment indicates this epitope does not reside in the side chains of Fr. C. On the other hand, the result with FAb 1 clearly indicates that two α-1,2-linked oligomannosyl side chains corresponding to Manα1-2Man and Manα1-2Manα1-2Man possess antigenic determinant of factors 1. In contrast, FAb 34 unable to recognize α-1,2-linked oligomannosyl side chain, whereas it recognized the side chain possessing terminal α-1,3-linked mannose, Manα1-3Manα1-2Manα1-2Man.

### Table 1. Assignment of chemical shifts of H1 signals of oligosaccharides obtained from *C. catenulata* mannan, Fr. C, by acetolysis.

<table>
<thead>
<tr>
<th>Oligosaccharide</th>
<th>Sugar residue$^a$</th>
<th>Chemical shift (ppm)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A(β)</td>
<td>A(β)</td>
</tr>
<tr>
<td>$M_2$</td>
<td>Man1-2Man(α)</td>
<td>5.047 5.350</td>
</tr>
<tr>
<td></td>
<td>Man2-Man(β)</td>
<td>5.139 4.893</td>
</tr>
<tr>
<td>$M_3$</td>
<td>Man1-2Manα1-2Man(α)</td>
<td>5.050 5.260 5.333</td>
</tr>
<tr>
<td></td>
<td>Man2-Man2-Man(β)</td>
<td>5.050 5.260 4.891</td>
</tr>
<tr>
<td>$M_4$</td>
<td>Man1-3Man1-2Man1-2Man(α)</td>
<td>5.144 5.041 5.264 5.337</td>
</tr>
<tr>
<td></td>
<td>Man2-Man2-Man2(β)</td>
<td>5.041 5.264 4.892</td>
</tr>
</tbody>
</table>

$^a$M denotes a mannose residue; $^b$This was measured at 45°C using acetone (2.217 ppm) as a standard; $^c$Configuration of reducing terminal residue.

### Table 2. Inhibition of agglutination of *Candida catenulata* cells with FAb 1, 9, and 34 by mannoooligosaccharides obtained from *C. catenulata* mannan, Fr. C.

<table>
<thead>
<tr>
<th>Oligosaccharide</th>
<th>Agglutination$^a$ with inhibitor amt (μmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2$^-1$ 2$^0$ 2$^1$ 2$^2$ 2$^3$ 0</td>
</tr>
<tr>
<td><strong>With FAb 1</strong></td>
<td></td>
</tr>
<tr>
<td>$M_1$</td>
<td>+3   +3  +3  +3  +3  +3</td>
</tr>
<tr>
<td>$M_2$</td>
<td>+2   +2  +2  +2  +2  +2</td>
</tr>
<tr>
<td>$M_3$</td>
<td>+1   +1  +1  +2  +3  +3</td>
</tr>
<tr>
<td>$M_4$</td>
<td>+2   +2  +3  +3  +3  +3</td>
</tr>
<tr>
<td><strong>With FAb 9</strong></td>
<td></td>
</tr>
<tr>
<td>$M_1$</td>
<td>+2   +2  +2  +2  +2  +2</td>
</tr>
<tr>
<td>$M_2$</td>
<td>+2   +2  +2  +2  +2  +2</td>
</tr>
<tr>
<td>$M_3$</td>
<td>+2   +2  +2  +2  +2  +2</td>
</tr>
<tr>
<td>$M_4$</td>
<td>+2   +2  +2  +2  +2  +2</td>
</tr>
<tr>
<td><strong>With FAb 34</strong></td>
<td></td>
</tr>
<tr>
<td>$M_1$</td>
<td>+3   +3  +3  +3  +3  +3</td>
</tr>
<tr>
<td>$M_2$</td>
<td>+3   +3  +3  +3  +3  +3</td>
</tr>
<tr>
<td>$M_3$</td>
<td>+3   +3  +3  +3  +3  +3</td>
</tr>
<tr>
<td>$M_4$</td>
<td>+1   +1  +2  +2  +3  +3</td>
</tr>
</tbody>
</table>

$^a$Agglutination was scored from high (+3) to low (+1).
The chemical structure of the cell wall mannan obtained from C. catenulata IFO 0745 strain (Fr. C) and the recognition sites of factor antibodies 1, 9, and 34 were proposed as shown in Figure 3. The side chain distribution was calculated using the peak-dimensions in the gel-filtration profile of the acetylation products (Figure 3(a)). The molar ratios of tetraosyl side chain were distinctly lower than that previously calculated from the dimension of H-1 signals in the 'H-NMR spectrum of the same mannan (Figure 3(b)) [19]. The average length of side chains, 2.6, and the value of branching frequency, 77.1%, calculated from the peak-dimension of elution pattern of acetylates (Figure 1) were lower than comparison with those calculated by the signal dimension of NMR spectrum (average length: 3.0, branching frequency: 91.3%). These findings showed that the acetylation conditions make to cleave not only α-1,6 linkage of backbone but also non-reducing terminal part of the relatively longer α-linked side chains. In conclusion, although acetylation is useful for the preparation of the oligosaccharides corresponding to side chains as haptons of immunochemical or biological function, the NMR analysis without using harsh procedure is useful for the detailed analysis for the distribution of side chains in the parent mannan.

In the previous study [10,12], we demonstrated that the α-1,2-linked mammooligosaccharides and the oligosaccharides containing a non-reducing terminal α-1,3-linked mannose residue corresponding to the epitopes of antigenic factors 1 and 34, respectively. In this study, we could prepare three oligosaccharides corresponding to the antigenic factors 1 and 34, Manα1-2Man, Manα1-2Manα1-2Man, and Manα1-3Manα1-2Man, which were isolated from α-1,6-linked polymannosyl backbone of Fr. C by the selective cleavage method, acetylation. Though Fr. C reacted weakly with Fab 9, we could not find the oligosaccharide which functions as an antigenic epitope of this antibody (Table 2). This phenomenon can explain that the site of factor 9 antibody is α-1,6-linked polymannosyl backbone of yeast mannan in accordance with previous finding [12]. Namely, it is concluded that the most of mannose (M) released by acetylation arose from the backbone part that is not connected by the side chain.

REFERENCES


