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Anomeric Proton and Carbon (H1-C1) NMR Chemical Shifts of Antigenic Mannans Obtained from Pathogenic Yeast *Candida tropicalis*

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Abstract

On two dimensional maps of ^{1}H-^{13}C correlation spectroscopy (H-C COSY) analysis for the mannan of *Candida tropicalis*, nine cross peaks of anomeric proton and carbon were useful for the purpose of obtaining information on the chemical structure of this molecule. Namely, the mannans was comb-like structure constructed with the linear α-1,6-linked polymannnosyl backbone and several oligomannnosyl side chains composed of α-1,2-, α-1,3-, and β-1,2-linkages. Therefore, in the structural investigation of comb-like mannan, two-dimensional H-C COSY analysis is as useful as two-dimensional nuclear Hartmann-Hahn (HOHAHA) analysis.

Keywords

Candida tropicalis, Cell Wall Mannan, Comb-Like Structure, ^{1}H-^{13}C Correlation Spectroscopy, Anomeric Carbon Chemical Shift, α-1,3-Linked Mannose

1. Introduction

Most of the antigenic activity of pathogenic *Candida* yeasts is carried out by *N*-linked polysaccharides composed of mannose that cover the outermost layer of their cell walls [1]. Therefore, structural studies on cell wall mannan of pathogenic *Candida* yeast have been actively conducted for the purpose of diagnosing candidiasis and searching for target antigens for yeast species identification [2] [3] [4] [5].

Nuclear magnetic resonance (NMR) analysis plays a major role in the study of the chemical structure of *Candida* yeast cell wall mannan. In recent years, it has become possible to determine the approximate overall structure of a mannan simply by performing a two-dimensional homonuclear Hartmann-Hahn (2D-HOHAHA) analysis of intact mannan molecule [6] [7]. This is because the nuclear Overhauser effect (NOE) cross-peak of various intact mannans and/or derived manno-oligosaccharides were sequentially assigned in two-dimensional maps such as nuclear Overhauser enhancement and exchange spectroscopy (NOESY) by Shibata *et al.* [8] [9] [10] and Kobayashi *et al.* [11] [12]

Candida tropicalis is one of the species that is often clinically isolated as a deep-seated mycosis-causing yeast. The cell wall mannan of this yeast is basically a comb-like structure in which several side chains are linked to linear backbone composed of α-1,6-linked mannose residues [13]. There are two types of these side chains, one consisting of α-1,2- and β-1,2-linked mannose residues [6], and the other containing α-1,3-linked mannose residues in addition to these mannose residues [14].

In this short report, we note that information on the two types of mannan structures from *Candida tropicalis* can be fully analyzed by two-dimensional ^{1}H-^{13}C correlation (H-C COSY) spectroscopy, which is not inferior to two-dimensional HOHAHA.

2. Materials and Methods

Candida tropicalis NBRC 0199 and 1400 strains were obtained from the National Institute of Technology and Evaluation, Chiba, Japan. These strains were maintained on Sabouraud agar slants. Cultivation of two *C. tropicalis* strains and preparation of mannan were performed as described [13]. These strains were cultivated in Sabouraud liquid medium at 27˚C for 72 h on a reciprocal shaker.

Preparation of mannan was conducted by a combination of hot-water extraction and Fehling solution method [15]. The purified mannans obtained from the cells of the *C. tropicalis* NBRC 0199 and 1400 were designated Fr. 0199 and Fr. 1400, respectively.

^{1}H-NMR spectrum (internal acetone, 2.217 ppm) was measured with a Jeol JNM-GSX 400 spectrometer on solutions (3 - 10 mg sample/0.7mL) in D_2O at 70˚C [16]. ^{13}C-NMR spectrum (internal CD_3OD, 49.00 ppm) was measured with the same spectrometer on solutions (15 - 25 mg sample/0.7mL) in D_2O at 55˚C [17]. Two-dimensional H-C COSY was also recorded under the same conditions as for the ^{1}H- and ^{13}C-NMR spectra in accordance with previous description [18].

3. Results and Discussion

Eight anomeric H1-C1 cross-peaks on a H-C COSY two-dimensional map of Fr. 0199 (**Figure 1(a)** and **Table 1**) were assigned based on the previous report [6]. The presence of cross-peak 1, 2, and 7 indicates that mannan contain β-1,2-linked oligomannosyl side chains. Cross-peaks 5 or 3 correspond to the 2-O-substituted

or unsubstituted forms of the backbone in which *α*-1,2-linked mannose residues are polymerized, respectively. The existence of internal *α*-1,2-linked mannose residues was confirmed by the appearance of cross-peaks 8, and 9. The appearance of cross-peak 4 indicates the presence of a non-reducing terminal *α*-1,2-linked mannose residue of long side chain. On the other hand, in the two-dimensional map of Fr. 1400 (**Figure 1(b)** and **Table 1**), the appearance of an additional cross-peak 6 indicates the presence of non-reducing terminal *α*-1,3-linked mannose residue. Summarizing these analysis results, the overall structure of *C. tropicalis* NBRC 0199 and 1400 strain mannans can be proposed as shown in **Figure 2**.

Table 1. Identification of chemical shifts of ^{13}C-^{1}H COSY spectrum of Fr. 0199 and Fr. 1400.

Cross-Peak	Chemical shift (ppm)[a]		Sugar residue[b]
	H-1	C-1	
1	4.839	101.82	**M***β*1-2(M*β*1-2)
2	4.915	99.86	M*β*1-2**M*β***1-2M*β*1
3	4.915	100.31	*α*1-6**M***α***1-6
4	5.055	102.98	**M***α*1-2M
5	5.083	99.18	*α*1-6**M***α***1-6 | 2 M*α*1
6	5.145	102.86	**M***α*1-3M*α*1
7	5.145	100.81	(*β*1-2M)β1-2**M***α*1-2
8	5.244	101.39	(*α*1-2M)*α*1-2**M***α*1-2
9	5.276	101.39	M*α*1-2**M***α*1-2M*α*1

[a]Measured using acetone (2.217 ppm) as a standard; [b]M denotes a D-mannose residue.

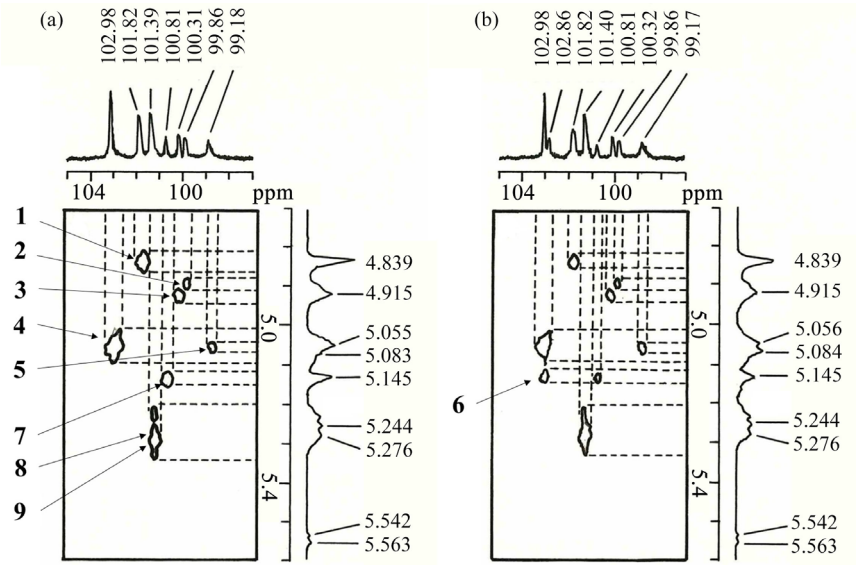
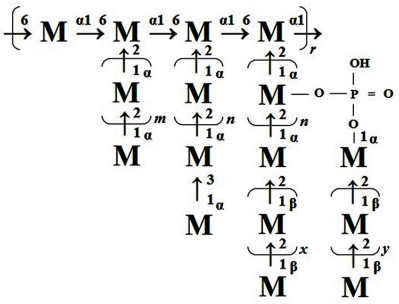


Figure 1. H-C COSY (anomeric region) spectra of Fr. 0199 (a) and Fr. 1400 (b).



Fr. 0199	+	+	−	+	+
Fr. 1400	+	+	+	+	+

Figure 2. Approximate overall structures of Fr. 0199 and Fr. 1400. M denotes a D-mannose. Polymerization of mannan unit *r* was not determined. The estimated polymerization degrees of the side chains were $m = 0 - 4$, $n \geq 2$, $x \geq 1$ and $y \geq 0$, respectively.

In the previous study [18], it was shown to be suitable to analyze the comb-like yeast mannan composed of α-1,2-, β-1,2- and α-1,6-linked mannose residues by two-dimensional H-C COSY. In this report, we have shown that this procedure is also useful in the structural analysis of similar mannan composed of α-1,2-, α-1,3-, β-1,2-, and α-1,6-linked mannose residues. However, in the single NMR analysis of undegraded mannan, the degrees of polymerization of the α-1,2- and β-1,2-linked mannose residues constituting the side chains (x and y values in **Figure 2**) cannot be identified. The only way to obtain such accurate information is to perform a hydrolysis method such as acetolysis on mannan and analyze the resultant oligosaccharides corresponding to the mannan side chains.

4. Conclusion

In the NMR analysis of yeast cell wall mannan, the H1-C1 cross-peaks of H-C COSY two-dimensional map give almost the same value of information as the H1-H2 cross-peaks of two-dimensional HOHAHA map. However, if there is a need to measure the degree of polymerization of the side chains in the mannan molecules, since not enough two-dimensional NMR analysis, will occur need to perform a partial acid-hydrolysis such as acetolysis on the mannan.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

NMR: Nuclear magnetic resonance

H-C COSY: ^{1}H-^{13}C correlation spectroscopy

HOHAHA: Nuclear Hartmann-Hahn

NOE: Nuclear Overhauser effect

NOESY: Nuclear Overhauser enhancement and exchange spectroscopy