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Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Various Yeasts as Aqueous Suspensions

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Carbon-13 nuclear magnetic resonance spectra of several yeasts were measured as aqueous suspensions, and were compared with those of the yeast mannan and the glucan. The spectrum of *Saccharomyces cerevisiae* was quite similar to the sum of the spectra of the mannan and the glucan. The signals attributable to the mannan were stronger than those of the glucan. The spectra of other yeasts, such as *Candida utilis*, *Candida pseudotropicalis*, and *Kluyveromyces fragilis* were different from each other. The results suggest that (1) this approach may be useful for chemotaxonomical examination of yeasts, (2) mannan and glucan in the cell wall showed a mobility similar to that of the pure preparations.

Keywords——carbon-13 NMR; yeast; mannan; zymosan; chemotaxonomy; immunomodulator

Introduction

Recently, we showed that the glucans contained in the fruit body of *Grifola frondosa* (*G. frondosa*) showed carbon-13 nuclear magnetic resonance (^{13}C-NMR) signals, measured as an aqueous suspension, similar to those of the extracted glucan fractions.[1a] These results also suggest that the mobility of glucans in the mushroom is similar to that in the extracts. Further, we reported that several mushrooms belonging to Basidiomycotina showed quite similar spectra to *G. frondosa*.[1b]

Yeast is an important fungus in various fields, such as fermentation, genetic engineering, immunomodulation, and opportunistic infection. This report is concerned with the application of ^{13}C-NMR spectroscopy to aqueous suspensions of various yeasts.

Materials and Methods

Tested Yeasts——*Saccharomyces cerevisiae* (*S. cerevisiae*), *Candida utilis* (*C. utilis*), *Candida pseudotropicalis* (*C. pseudotropicalis*), *Kluyveromyces fragilis* (*K. fragilis*), and brewer's bottom yeast were purchased from Sigma Chem. Co., Ltd.

Other Materials——Zymosan A and mannans (precipitated by the Fehling or cetavlon method) were purchased from Sigma Chem. Co., Ltd. Laminarin was from Nakarai Chem. Co., Ltd. *G. frondosa* was a gift from Nippon Beet Sugar Mfg. Co., Ltd. Grifolan was prepared as described previously.[1]

Preparation of the Defatted Yeasts——Each yeast was suspended in 80% aqueous ethanol and the suspension was refluxed for 30 min. This procedure was repeated several times. After air drying, the yeast was washed with water and then lyophilized.

^{13}C-NMR Spectral analysis——^{13}C-NMR spectra were measured in a 10ϕ tube and recorded at room temperature for aqueous suspensions (several drops of D_2O were added to provide a lock signal) with a JEOL FX-200 (for carbon-13 at 50.1 MHz) spectrometer. The spectra were obtained in the pulsed Fourier-transform mode with complete proton decoupling.

About 300 to 500 mg of a yeast was suspended in 3 ml of H_2O. Representative measurement conditions were as follows: pulse width (PW 1) 15 μs, data points (POINT) 8192, observation frequency width (FREQU) 12004 Hz, sampling time (ACQTM) 341.1 ms, pulse delay (PD) 100 ms, center frequency offset (OBSET) 83.8 kHz, irradiation frequency (IRFRQ) 199.5 MHz, irradiation center offset (IRSET) 57.7 kHz.

Other Methods——Other methods used in this paper were performed as described previously.[1]

Results

^{13}C-NMR Spectrum of *S. cerevisiae* as an Aqueous Suspension

Figure 1a shows the ^{13}C-NMR spectrum of *S. cerevisiae* as an aqueous suspension. The spectrum showed signals attributable to both low- and high-molecular-weight (Mw) carbohydrates. The low-Mw carbohydrate (signals at 94.2, 74.0, 73.8, 73.2, 72.0, 62.0) was assigned as α,α-trehalose by comparing the spectrum with that of an authentic commercial sample.

Figure 1b shows the ^{13}C-NMR spectrum of washed and defatted *S. cerevisiae* for visualizing the polymer signals. This spectrum is well resolved and different from the spectra of mushrooms previously reported.[1)]

In order to identify these polymer signals, they were compared with reported values[2)] and spectra of yeast mannans (Figs. 1c, d), zymosan (Fig. 1e), laminaran (Fig. 1f; β-1,3, β-1,6-glucan) and *G. frondosa* as an aqueous suspension (Fig. 1g). Mannan signals and glucan signals in *S. cerevisiae* were differentiated by comparison with the spectra of mannans and zymosan. The major signals observed in the ^{13}C-NMR spectrum of *S. cerevisiae* were assigned as shown in Table I. Most of the signals of anomeric carbons (A,B,C,E) and ring carbons (F,G,H,I,K,L) were not overlapped in the cases of mannans and glucans. These results suggest that mannans and glucans in the yeast would be observable by ^{13}C-NMR spectroscopy and showed some mobility in the cell wall.

Comparison of the ^{13}C-NMR Spectra of Various Yeasts

Chemotaxonomical examinations of yeasts by using NMR spectroscopy were performed

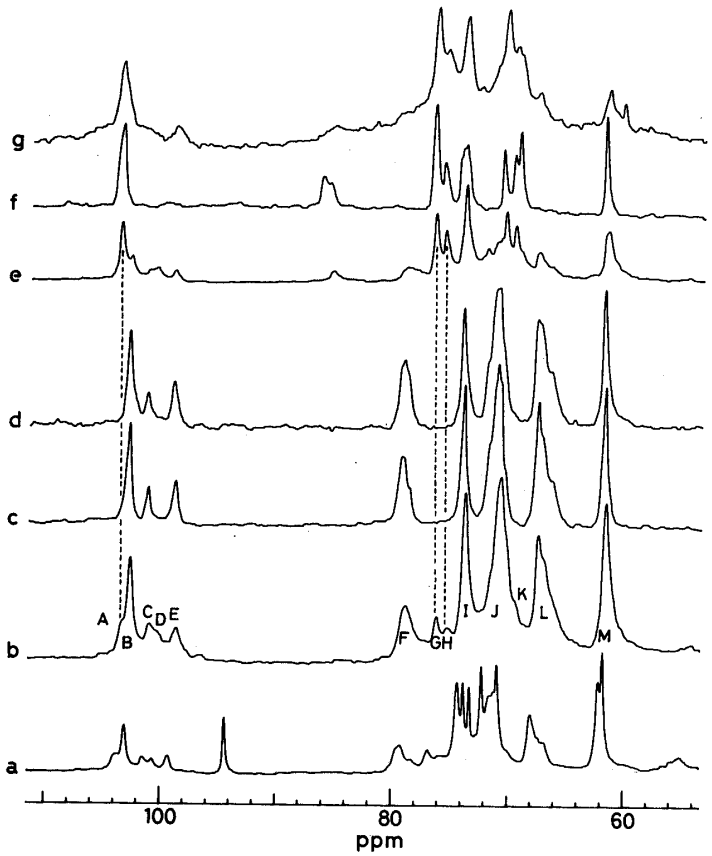

Fig. 1. Carbon-13 NMR Spectrum of *S. cerevisiae* as an Aqueous Suspension

a, *S. cerevisiae*; b, *S. cerevisiae* after defatting treatment; c, yeast mannan (Fehling method); d, yeast mannan (Cetavlon method); e, zymosan; f, laminaran; g, amylase treated *G. frondosa* as aqueous suspension.

TABLE I. Assignment of ^{13}C-NMR Signals of *S. cerevisiae*

	Chemical shift	Mannan/ glucan	Assignment
A	103.5	Glucan	C-1
B	103.0	Mannan	C-1 of non-reducing end and 3-*O*-substituted residues
C	101.5	Mannan	C-1 of 2-*O*-substituted residues
D	100.5		Unknown
E	99.3	Mannan	C-1 of 2,6-di-*O*-substituted residues
F	*ca.* 79.5	Mannan	C-3 of 3-*O*-substituted, C-2 of 2-*O*-substituted, and C-2 of 2,6-di-*O*-substituted residues
G	*ca.* 76.5	Glucan	C-3 of unsubstituted residues and C-5
H	*ca.* 75.5	Glucan	
I	*ca.* 74.0	Mann/gluc	C-5 of mannan and C-2 of glucan
J	*ca.* 70.0	Mannan	C-2 and C-3 of unsubstituted residues
K	*ca.* 69.5	Glucan	C-4 of unsubstituted and C-6 of 6-*O*-substituted residues
L	*ca.* 68.0	Mannan	C-4
M	*ca.* 62.0	Mann/gluc	C-6

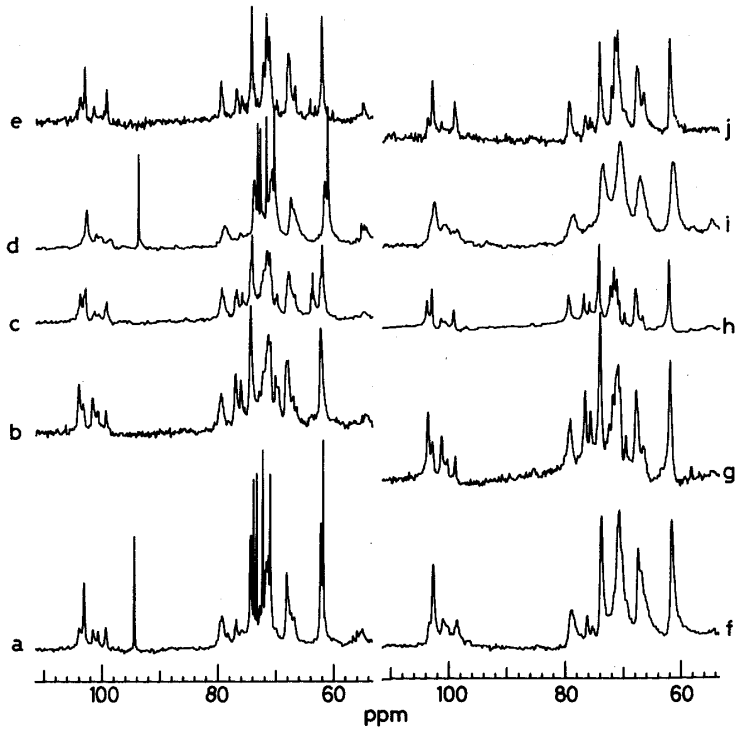

Fig. 2. Carbon-13 NMR Spectra of Aqueous Suspensions of Various Yeasts

a, *S. cerevisiae*; b, *C. utilis*; c, *C. pseudotropicalis*; d, brewer's bottom yeast; e, *K. fragilis*; f—j, defatted yeast of a to e, respectively.

by Gorin *et al.*,[3] Fukazawa *et al.*,[4] and Shinoda *et al.*[5] They used mannans prepared by aqueous extraction for making the comparisons. In order to classify yeast cell wall components more easily, we applied NMR spectroscopy to the yeasts as aqueous suspensions. Commercially available yeasts were used. Figures. 2a—e showed the spectra of native yeasts. *S. cerevisiae* (Fig. 2a) and brewer's bottom yeast (Fig. 2d) showed signals due to α,α-trehalose. Figures 2f—j shows the spectra of defatted and washed yeasts. In comparison with the signals of *S. cerevisiae*, as shown in Table I, the spectra showed the following characteristics. (1) From the ratio of signals A/B and G+H/F, *C. utilis* (Fig. 2h) and *C. pseudotropicalis* (Fig. 2g) contained more glucan than *S. cerevisiae*. (2) From the intensities

of anomeric carbons B, C, and E, the structures of mannans in *C. utilis* (Fig. 2h), *C. pseudotropicalis* (Fig. 2g), and *K. fragilis* (Fig. 2j) were different from that of *S. cerevisiae* mannan.

Discussion

In this paper, it is shown that yeast cell wall components, such as mannan and glucan, can be detected by ^{13}C-NMR spectroscopy of aqueous suspensions of the yeast cells. Furthermore, this method can be used to gain some information on the mannan structure. Consequently, this approach could be useful for studies on the chemotaxonomy and biosynthetic characteristics of yeasts. Chemotaxonomical examination of yeasts is important. Some comparative studies of mannan structure by NMR spectroscopy have been reported.[3-5] It is known that proton nuclear magnetic resonance (^{1}H-NMR) is more useful than ^{13}C-NMR spectroscopy for chemotaxonomical examination,[3] because some signals in the anomeric region (such as signal B in Table I) overlap in the ^{13}C-NMR spectra. Unfortunately, ^{1}H-NMR spectroscopy of the aqueous suspensions was unsuccessful because of the low resolution (data not shown). Therefore, the method presented in this paper should be mainly useful for monitoring the mannan structure, except for the limitation of several signal overlappings.

Biosynthesis of the yeast mannoprotein in *S. cerevisiae* has been precisely examined.[6] In that work, several mutants deficient in several parts of mannan structure were used. The method presented in this paper should be easily applicable to such mannan structures, and should therefore be useful for studying the biosynthesis of mannans in yeasts.

Yeast glucan is known to be composed of β-1,3- and β-1,6-glucosidic linkages. Under physiological conditions, β-1,3-glucan forms a gel and shows less well resolved signals, especially for the β-1,3-linked main chain. In the case of yeast and zymosan, the signals at 86 ppm, assigned to C-3 of β-1,3-linked glucosyl units, could hardly be observed. This suggests that the main chain unit of yeast glucan has rather low mobility, as with lentinan[7] and grifolan.[1]

The line width of each signal should be related to the mobility of polysaccharides in the solvent. Previously, we showed that the glucans in the mushroom possess relatively high mobility.[1a,b] The present results suggest that the mannans and glucans in the cell wall show similar mobility to the preparations. Mannans and glucans are well known immunomodulators. Thus, the biological activity of mannans and glucans may reflect that of the yeast cells.

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