Isolation and Identification of a Choline-linked Mannobiose in the Glycoproteins of *Fusarium* sp. M7-1

Shojiro Iwahara,* Nahoko Suemori, and Kaoru Takegawa

Department of Bioresource Science, Faculty of Agriculture, Kagawa University, Miki-cho, Kagawa 761-07, Japan

Received August 11, 1995

An unidentified oligosaccharide was isolated from an oligomer mixture derived by alkaline borohydride treatment from glycoproteins of *Fusarium* sp. M7-1. The isolated compound was identified as O-2,6-Mannopyranosyl (1→2)-α-Mannitol-6-phosphocholine by NMR and MS spectrometry.

Key words: *Fusarium*; glycoproteins; choline; mannobiose

In previous papers, we reported the chemical structures of various kinds of O-glycosidically linked carbohydrate chains in the glycoproteins of *Fusarium* sp. M7-1. The presence of choline-linked oligosaccharide units in the polysaccharide chain was also demonstrated in a previous paper. During the course of these studies we have found that choline-linked oligosaccharide is present in the glycoproteins as a carbohydrate chain. This paper describes the isolation and identification of the oligosaccharide.

The oligosaccharide mixture was obtained from the glycoproteins by alkaline borohydride treatment as described. The oligosaccharide mixture (100 mg of total sugar in 100 ml of deionized water, pH 8) was poured into a column containing Dowex 1 × 2 (formate, 1.5 × 30 cm) and eluted with deionized water. The eluate was concentrated to a small volume. The concentrate was chromatographed on a column of Bio-Gel P-4 (3 cm × 90 cm) eluted with deionized water at a flow rate of 1 ml per min at 60°C. The same elution profile was observed as described previously. During the purification process it was found that an unidentified compound was contained in the fractions eluted between the tetramer and pentamer (elution volume of 700–800 ml). The unidentified-compound-containing fractions were combined and concentrated to a small volume. The concentrate was further purified by gel filtration on Bio-Gel P-4 and HPLC on Hitachi GL-C611 and Shim-pack ION S 802. Thus, about 20 mg of the purified sample was obtained from 500 mg of the oligosaccharide mixture.

The isolated compound (10 mg) was hydrolyzed with 2 M of trifluoroacetic acid (TFA) in a sealed tube at 100°C for 3 h. The TFA was removed by extraction with ethyl acetate, then the hydrolyzate was passed through a column of Dowex 50 (H+).

![Fig. 1. 1H-NMR Spectrum of the Isolated Compound.](image)

The spectrum was recorded on a JEOL-GSX 400 spectrometer at 400 MHz operating in Fourier transform mode at a probe temperature of 28°C. Chemical shifts were measured indirectly relative to acetone in D$_2$O ($δ=2.25$ ppm).

* To whom correspondence should be addressed.
and the column was washed with deionized water. The eluate was passed through a column of Dowex 1 × 2 (Cl⁻, 1 × 5 cm) with deionized water. The eluate was concentrated to a small volume (neutral sugar fraction). The acidic component retained on the Dowex 1 × 2 column was eluted with 1 M NaCl. The salt was removed by gel filtration on Bio-Gel P-2. The sugar-containing fractions were combined and lyophilized (compound A). The basic component retained on the Dowex 50 column was eluted with 2 M HCl and the choline-containing fractions were combined, concentrated, and lyophilized (compound B). Analysis of the neutral sugar fraction showed that the fraction consisted of mannose and a trace of mannotol. The ¹H- and ¹³C-NMR spectra of compound A (data not shown) are identical with those of mannotol-6-phosphate. The ¹H-NMR spectrum of compound B (data not shown) is identical with that of choline. Furthermore, compound B was oxidized by choline oxidase. Therefore, compound B was identified as choline. The content of each component in the hydrolysate was analyzed by the method described previously. The result showed that these components are present in an equimolar amount. The ¹H-NMR spectrum of the isolated compound is shown in Fig. 1. The main signals were assigned by ¹H-¹H COSY (data not shown) and by analogy with reported compounds.

In the ¹H-NMR spectrum, the signals other than those of choline are identical with those of Manz₁→2Man-ol. In the NOE difference spectrum of the isolated compound (data not shown), NOE was observed at the C-2 proton of mannotol with irradiation at the H-1 signal of the mannotose residue, indicating that the mannotose residue is linked to the C-2 of mannotol. The data of the chemical shift (5.01 ppm) and J value (2.0 Hz) of the H-1 signal of mannotose residue and NOE difference spectrum indicated that mannotose is linked to C-2 of mannotol through an α-linkage. It can be concluded that choline is linked to C-6 of mannotol through a phosphodiester linkage based on the following criteria: (1) acid hydrolysis of the compound gave mannotol-6-phosphate, (2) in ¹³C-NMR spectrum (data not shown) four split signals caused by coupling with phosphorus were observed at 60.8 ppm (C-1 of choline), 47.8 ppm (C-2 of choline), 66.2 ppm (C-6 of mannotol), and 76.4 ppm (C-5 of mannotol). From these data mentioned above it is concluded that the isolated compound has the structure illustrated in Fig. 1. The Fab-Ms spectrum (Fig. 2) also supports the proposed structure.

In this study we demonstrated the presence of choline-linked mannotose as one of the oligosaccharide chains in the glycoproteins of Fusarium sp. M-7.1. The presence of choline-linked oligosaccharide units in the polysaccharide chain of Fusarium sp. M-7-1 has been demonstrated in a previous paper. The presence of choline-linked mannosyl residues in the mannian region of galactofuranosyl polysaccharide chain in the glycopeptide of Penicillium charlesii has also been reported.

References