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P.M.R. STUDIES ON THE ANTITUMOUR POLYSACCHARIDE ISOLATED FROM *Coriolus versicolor*

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ABSTRACT

Application of p.m.r. spectroscopy to the antitumour glycoprotein extracted from mycelia of *Coriolus versicolor* showed that the polysaccharide (D-glucan) chain contains (1→3)-α, (1→4)-α, (1→6)-α, (1→3)-β, (1→4)-β, and (1→6)-β linkages. The assignments of the characteristic peaks to amino acid residues in the protein portion are briefly discussed.

INTRODUCTION

Various polysaccharides of plant, fungal, and bacterial origin strongly inhibit the growth of transplantable tumours in animals. Thus, lentinan[1,2] (glucan) isolated from *Lentinus edodes* (Berk.) Sing. has considerable activity against sarcoma 180, and a polysaccharide obtained[3] from the mycelium of *Coriolus versicolor* inhibits the growth of this tumour implanted subcutaneously in mice. Tsukagoshi and Ohashi[4] showed that a glycoprotein isolated from *Coriolus versicolor* (Fr.) Quél of Basidiomycetes contains ~15% of tightly bound protein, and strongly inhibits the growth of mouse sarcoma 180 and rat ascites hepatoma AH-13 when administered orally. Miyazaki *et al.*[5] reported that an extracellular, antitumour glucan obtained from the culture filtrate of *Coriolus versicolor* had a highly branched structure including (1→3) and (1→6) linkages. Recently, Saito *et al.*[6] showed that an antitumour glucan (HA-3) obtained from *P. ostreatus* contains (1→3)-β and (1→4)-α linkages.

We now report on the p.m.r.-spectral data for the antitumour glycoprotein isolated from mycelia of *Coriolus versicolor* (Fr.) Quél of Basidiomycetes, as a contribution to the determination of structure–activity relationships. The signals from anomeric protons are usually readily distinguished, and have been used for the detection and quantification of α and β anomers and various types of glycosidic linkages in mono-, oligo-, and poly-saccharides[7–12].

RESULTS AND DISCUSSION

The ^{1}H-n.m.r. spectrum (D_2O, 90°) of polysaccharide-I, isolated from mycelia of *Coriolus versicolor* (Fr.) Quél of Basidiomycetes by extraction with hot water, is illustrated in Fig. 1. The spectral features were essentially invariant in the temperature

range from 25–90°, except for the expected shift to lower field of the resonance of residual protons in the solvent, and the line broadening of all resonances at lower temperatures. One main part of this spectrum (0–3 p.p.m.) contains resonances for CH, CH_2, and CH_3 protons of the amino acid residue in the protein moiety. The second main region (3–6 p.p.m.) contains the resonances for the CH and CH_2 protons of the polysaccharide portion, and the α-CH protons of the amino acid residues (3–4.5 p.p.m.)[13,14]. The data in Fig. 1 confirm that polysaccharide-I is a glycoprotein, as reported by Tsukagoshi and Ohashi[4]. G.l.c. analysis of a hydrolysate of the polysaccharide portion of polysaccharide-I revealed mainly D-glucose[4]. By comparison with the chemical shifts[8,9,15] of D-gluco-oligosaccharides and -polysaccharides for solutions in D_2O, the region 4.4–5.5 p.p.m. in Fig. 1 may be assigned to anomeric protons. The intensities of signals for α-CH protons of amino acid residues also present in this region are small, because polysaccharide-I contains[4] only ~15% of protein, and α-CH protons resonate[15] at 3.0–4.5 p.p.m. The presence of several peaks in the range 4.4–5.5 p.p.m. indicates a variety of glycosidic linkages. On the basis of literature data[8,9,15], the four main peaks (p.p.m.) are assigned to linkages as follows: 5.39, (1→4)-α and/or (1→3)-α; 5.00, (1→6)-α; 4.75, (1→3)-β; and 4.55, (1→4)-β and/or (1→6)-β. These assignments are tentative and made on the assumption that the polysaccharide is essentially a glucan[4].

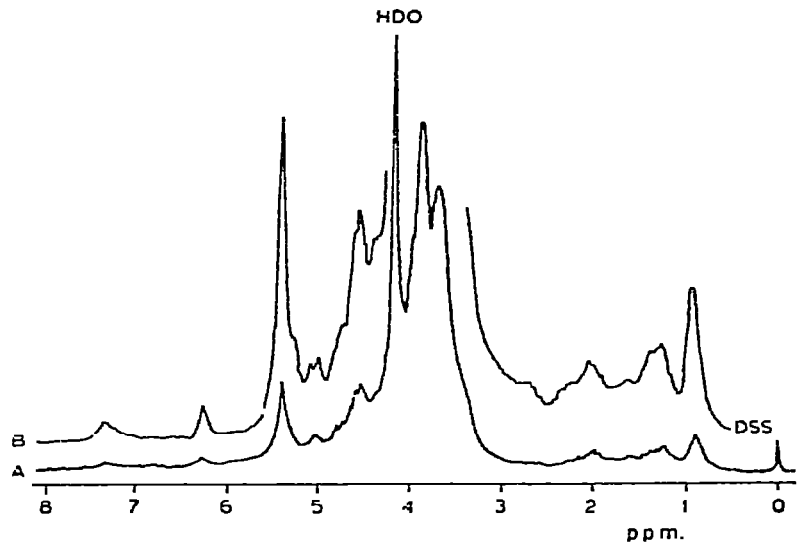

Fig. 1. ^{1}H-N.m.r. spectra of a 10% solution of polysaccharide-I in D_2O at 100 MHz and 90°: A, single scan; B, 64 accumulations.

From these assignments, the apparent percentage of different types of linkages can be estimated, and the results are shown in Table I. There was a small effect on these data by variation of conditions of sample preparation, including cultivation.

TABLE I

CHEMICAL COMPOSITION OF THE POLYSACCHARIDE PORTION OF THE ANTITUMOUR, PROTEIN-BOUND POLYSACCHARIDE ISOLATED FROM MYCELIA OF *Coriolus versicolor*

	Composition (%)				*αβ-Ratio*
	(1→3)-α and/or (1→4)-α	*(1→6)-α*	*(1→3)-β*	*(1→4)-β and/or (1→6)-β*	
Polysaccharide-I	42	7	10	41	49.51
Polysaccharide-II	92	8	0	0	100:0

The ^{1}H-n.m.r. spectrum (Fig. 2) of polysaccharide-II, isolated from polysaccharide-I by column chromatography, showed it to contain mainly α linkages, *i.e.*, (1→3)-α and/or (1→4)-α (5.39 p.p.m.), and (1→6)-α (5.00 p.p.m.). The apparent composition of polysaccharide-II is also shown in Table I. The result is consistent with the generally accepted fact that α-D-glucans are more soluble than the β isomers.

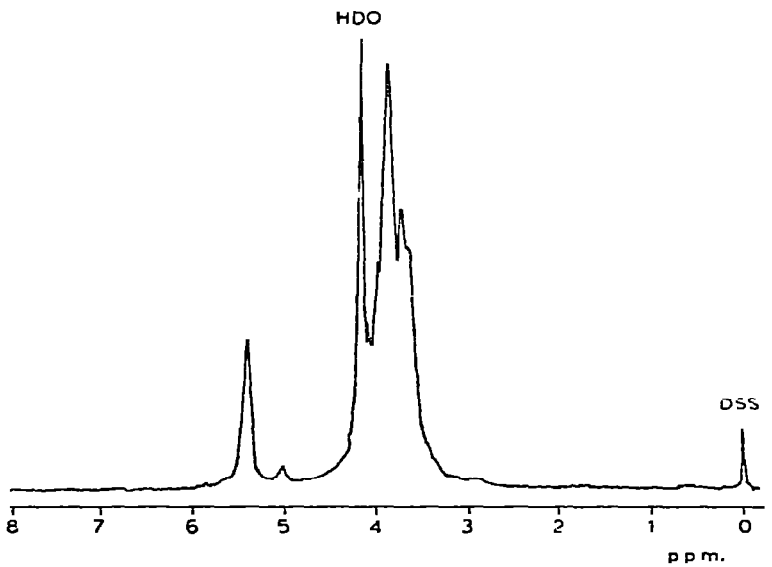

Fig. 2. ^{1}H-N.m.r. spectrum of a 10% solution of polysaccharide-II in D_2O at 100 MHz and 90°.

The ^{1}H-n.m.r. spectra (Fig. 3) of permethylated polysaccharide-II and amylose are essentially identical, except for the small signal at 4.95 p.p.m which corresponds to H-1 of (1→6)-α-linked permethylated oligosaccharides[10,11]. Thus, the main portion of polysaccharide-II may be amylose-like with (1→4)-α-D linkages, although ~8% of (1→6)-α-D linkages are also present. This result indicates that the resonance at 5.39 p.p.m. in Fig. 1 may be mainly due to H-1 in (1→4)-α-linked D-glucose residues.

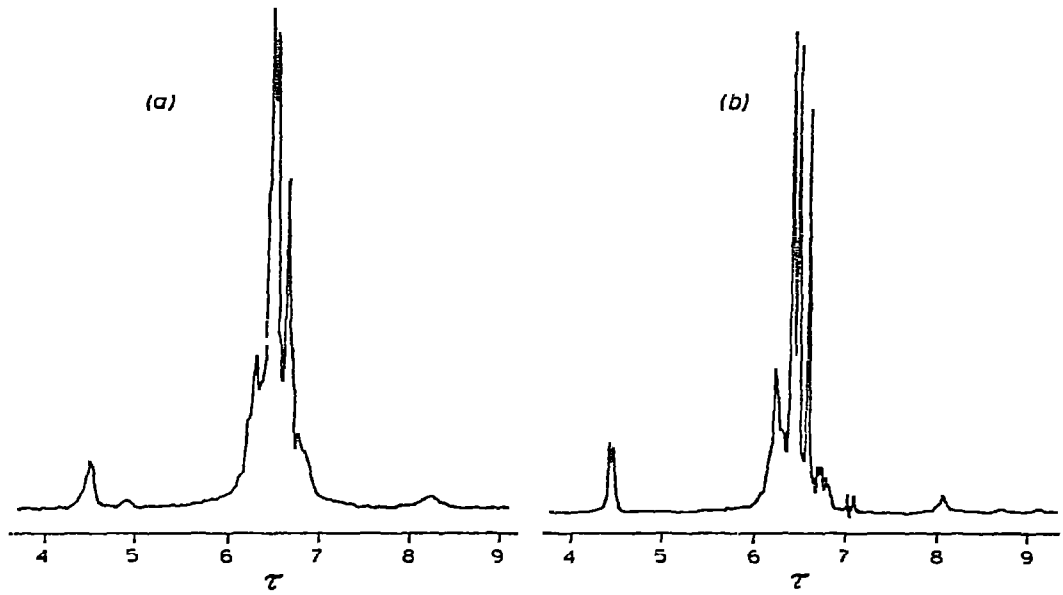

Fig. 3. ^{1}H-N.m.r. spectra of 10% solutions of (*a*) permethylated polysaccharide-II and (*b*) permethylated amylose in $CDCl_3$ at 100 MHz and 70°.

By comparison with the ^{1}H-n.m.r. spectra of lysozyme[14], the signals in the region 0–3 p.p.m. in Fig. 1 may be assigned as follows: 0.89, methyl protons of leucine, isoleucine, and valine residues; 1.23, methyl protons of threonine, 1.41, mainly methyl protons of alanine; 1.66, side-chain protons of lysine, arginine, and leucine residues; 2.06, methyl protons of methionine residues. The relative intensities of the peaks appearing in this region are roughly compatible with the results of amino acid analysis.[4]. The resonances appearing at lower field (6–8 p.p.m.) arise mainly from aromatic protons and from residual NH protons in the amino acid residues.

The results presented indicate the structural complexity of the glycoprotein isolated from mycelia of *Coriolus versicolor* (Fr.) Quél of Basidiomycetes. A similarity between polysaccharide-I and the polysaccharide HA-3 obtained from *P. ostreatus*[6] is that both contain (1→4)-α and (1→3)-β-linked D-glucose residues.

EXPERIMENTAL

Materials. — The polysaccharides were isolated and purified as described in the literature[4]. Polysaccharide-I was isolated from mycelia of *Coriolus versicolor* (Fr.) Quél of Basidiomycetes by extraction with hot water; the protein and polysaccharide portions are chemically linked[4], and the latter (~85%) contains mainly D-glucose residues together with minor amounts of mannose and xylose residues[4].

Elution of polysaccharide-I from a column of DEAE-cellulose with hot water gave polysaccharide-II as the most soluble fraction. The polysaccharide-II was methylated by Hakomori's procedure[16].

Method. — ^{1}H-N.m.r. spectra of polysaccharides were obtained with a JEOL JNM PS-100 spectrometer operated at 100 MHz. Samples were subjected to deuterium exchange by repeated dissolution in D_2O and freeze-drying, and then examined as 10% solutions in D_2O. Sodium 4,4-dimethyl-4-silapentane-1-sulphonate (DSS) and tetramethylsilane (Me_4Si) were used as internal standards for solutions in D_2O and $CDCl_3$, respectively. Signal intensities obtained by integration are accurate to $\pm 3\%$. The effective signal-to-noise ratio was improved by means of the multiscan average technique with a JEOL EC-6 computer.

REFERENCES

1 Y. Maeda and G. Chihara, *Nature (London)*, 229 (1971) 634.
2 Y. Maeda, J. Hamuro, and G. Chihara, *Int. J. Cancer*, 8 (1971) 41–46.
3 S. Naruse, K. Fujii, and H. Ito, *Proc. Japan Cancer Assoc., 30th Ann. Meeting*, 1971, p. 171.
4 S. Tsukagoshi and F. Ohashi, *Gann*, 65 (1974) 557–558.
5 T. Miyazaki, T. Yadomae, M. Sugiura, H. Ito, K. Fujii, S. Naruse, and M. Kunihisa, *Chem. Pharm. Bull.*, 22 (1974) 1739–1742.
6 H. Saito, Y. Yoshioka, and F. Fukuoka, Paper read at the 14th NMR Discussional Meeting, Fukuoka, Japan, October 1975.
7 R. U. Lemieux and J. D. Stevens, *Can. J. Chem.*, 44 (1966) 249–262.
8 T. Usui, M. Yokoyama, N. Yamaoka, K. Matsuda, K. Tujimura, H. Sugiyama, and S. Seto, *Carbohydr. Res.*, 33 (1974) 105–116.
9 J. N. C. Whyte, *Carbohydr. Res.*, 16 (1971) 220–224.
10 D. E. Minnikin, *Carbohydr. Res.*, 23 (1972) 139–143.
11 N. Frank, H. Friebolin, G. Keilich, J. P. Merle, and E. Siefert, *Org. Magn. Resonance*, 4 (1972) 725–732.
12 J. N. Kamerling, M. J. A. De Bie, and J. F. G. Vliegenthart, *Tetrahedron*, 28 (1972) 3037–3047.
13 G. C. K. Roberts and O. Jardetzky, *Adv. Protein Chem.*, 24 (1970) 447–545.
14 C. C. McDonard, W. D. Phillips, and J. D. Glickson, *J. Am. Chem. Soc.*, 93 (1971) 235–246.
15 W. M. Pasika and H. Cragg, *Can. J. Chem.*, 41 (1963) 293–299.
16 S. Hakomori, *J. Biochem.*, 55 (1964) 205–208.