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Note

A β-D-glucan isolated from the fruiting bodies of *Hericium erinaceus* and its aqueous conformation

Qun Dong,* Lian-meng Jia and Ji-nian Fang

Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Science, Graduate School, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Shanghai 201203, China

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Abstract—HEP3, a β-D-glucan slightly soluble in water, was isolated from the alkaline extract of the fruiting bodies of *Hericium erinaceus*. Its chemical structure was investigated by methylation analysis, periodate oxidation, Smith degradation and by IR and NMR spectroscopy. It was shown to have a main chain composed of β-(1→3)-linked D-glucopyranosyl residues, with single unit glucosyl branches attached to O-6 of every third backbone residue. Viscometry and Congo red reaction indicated that HEP3 has a highly ordered hydrogen-bond dependent conformation in aqueous solution, which collapses in strong alkaline solution.

Keywords: *Hericium erinaceus*; β-(1→3)-D-Glucan; Chemical structure; Conformation

Hericium erinaceus (Bull. ex Fr.) Pers (lion's mane) is an edible basidiomycetous fungus (Hydnaceae). Its fruiting bodies are well known as a traditional Chinese medicine or food and have attracted much investigation due to their health effects when used as a home remedy for gastric and duodenal ulcers and some other diseases.[1] This fungus contains polysaccharides, which exhibit immunomodulating activity and antiradiative effects.[2,3] A rhamnoglucogalactan fraction has been isolated from the boiling water extract and structurally characterized.[4] As we know, branched (1→3)-β-D-glucans are common components of the cell wall of many basidiomycetous fungi and are often reported to show immunomodulatory activity.[5,6] In this communication, we describe a branched (1→3)-β-D-glucan, designated HEP3, isolated from the fruiting bodies of *H. erinaceus*. Its aqueous conformation is revealed by the viscometric method and complex formation with Congo red in alkaline solution.

HEP3 was obtained from the fruiting bodies of *H. erinaceus* in a yield of ca. 0.2% on the basis of the crude material, using alkali extraction and anion-exchange chromatography on a DEAE-cellulose column. On HPGPC, HEP3 showed a symmetrical peak, indicating a homogenous fraction. The average molecular weight was estimated to be higher than 1.0×10^6 g/mol. After complete hydrolysis with 2 M trifluoroacetic acid (TFA), TLC analysis showed that the polysaccharide contains no uronic acid. GLC analysis indicated that it was composed exclusively of glucose. The absorption at 890 cm^{-1} in the IR indicated that HEP3 has β-glucopyranosidic linkages, which was further supported by its low $[\alpha]_{20}^{D}$ value of +14.6 (*c* 0.29, H_2O).

The ^1H NMR spectrum (not shown) of HEP3 displayed only one anomeric signal at δ 4.6 ppm, and the ^{13}C NMR spectrum contained two anomeric signals at δ 103.46 ppm and δ 102.97 ppm, further indicating a β-anomeric configuration for glucopyranosyl units.[7] The signals at δ 86.72 and δ 86.0 ppm arose from the substituted C-3 of glucose. The signal at δ 69.02 ppm was assigned to C-6 of branched (1→3)-β-D-glucosyl residues, as was supported by the corresponding

* Corresponding author. Tel.: +86 21 5080 6600; fax: +86 21 5080 7088; e-mail: dongqun@hotmail.com

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Table 1. ^{13}C NMR spectral assignments of HEP3 in D_2O–NaOD[a]

Residues	C-1	C-2	C-3	C-4	C-5	C-6
β-D-Glc*p*(1→	102.97	75.00	76.11	70.04	76.11	60.96
→3)-β-D-Glc*p*(1→	103.46	73.60	86.72	68.42	76.11	60.96
→3,6)-β-D-Glc*p*(1→	103.46	73.60	86.00	68.42	75.00	69.02

[a] Concentration, 30 mg/0.5 mL.

Table 2. GC–MS data for methylation analysis of HEP3

Methylated sugars	Linkage types	Molar ratios (mol %)	Major mass fragments (*m*/*z*)
2,3,4,6-Me_4-Glc	Terminal	23.9	45, 71, 87, 101, 117, 129, 145, 161, 205
2,4,6-Me_3-Glc	1,3-	50.5	45, 58, 71, 87, 99, 101, 117, 129, 161, 201, 233
2,4-Me_2-Glc	1,3,6-	25.6	58, 87, 99, 101, 117, 129, 139, 159, 189, 201, 233

reversed peak in the DEPT spectrum (not shown). Other ^{13}C NMR signals were tentatively assigned and are shown in Table 1, referred to the literature values.[8]

After methylating three times using the modified Ciucanu method, the OH absorption at 3600–3200 cm^{-1} in IR disappeared, indicating the completeness of methylation. The permethylated polysaccharide was depolymerized and converted into partially methylated alditol acetates. GC–MS analysis showed three types of linkages, corresponding to T-Glc*p* (terminal), 1,3-linked Glc*p* and 1,3,6-linked Glc*p*, respectively, approximately in the molar ratio of 1:2:1. The data indicated an O-6-branched (1→3)-β-D-glucan structure of HEP3 (Table 2).

In order to ascertain whether HEP3 has a single unit D-glucosyl or (1→3)-linked multiple unit side chains, periodate oxidation and Smith degradation were carried out. After 5 days of periodate oxidation, HEP3 consumed 0.5 mol of periodate and produced 0.27 mol of formic acid per mole of glucosyl residues. This result was in good accord with the theoretical values (0.5 mol of periodate and 0.25 mol of formic acid) calculated from methylation analysis. HEP3-SA, the polyol derivative from periodate oxidation, was composed of D-glucose and glycerol in a molar ratio of 2.9:1, also in agreement with the data above.

HEP3-SP, the Smith-degradation product of HEP3, showed six ^{13}C NMR signals, which were assigned clearly to the six carbons of (1→3)-linked β-D-glucosyl units: δ 103.37 ppm (C1), 73.54 ppm (C2), 86.61 ppm (C3), 68.55 ppm (C4), 76.42 ppm (C5), 61.00 ppm (C6). The disappearance of terminal and (1→3)- and (1→6)-linked Glc*p* in its linkage analysis further confirmed a linear structure for HEP3-SP. These results suggested that HEP3 contains branches of a single glucosyl unit, which were completely removed by Smith degradation (Fig. 1).

It could thus be concluded that HEP3 has a backbone of (1→3)-linked β-D-glucopyranosyl units, with one single unit β-D-glucopyranosyl branch substituted at O-6,

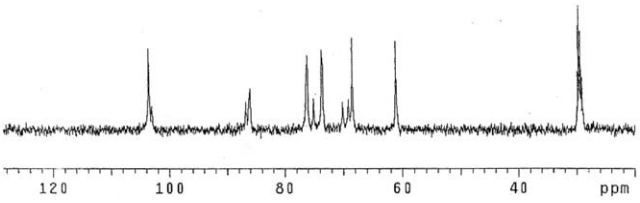

Figure 1. ^{13}C NMR spectrum of polysaccharide HEP3 isolated from *Hericium erinaceus*. The sample was dissolved in D_2O–NaOD (30 mg/0.5 mL) and determined at 30 °C, with acetone as the internal standard (δ 29.50 ppm).

on average, for every three backbone units (Fig. 2). This result was in agreement with other same types of β-D-glucans isolated from other fungi or lichens.[8–10]

It has been reported that many (1→3)-(1→6)-β-D-glucans adopt ordered helical conformations in aqueous solution.[11,12] A strong alkaline environment can induce the denaturation of such helical structures by breaking the intra- and intermolecular hydrogen bonds, leading to reduced aqueous viscosity.[13] As shown in Figure 3, significant reduction of intrinsic viscosity [η] was observed as NaOH concentration increases from 0 to 0.2 M, reflecting a continuous denaturation process of

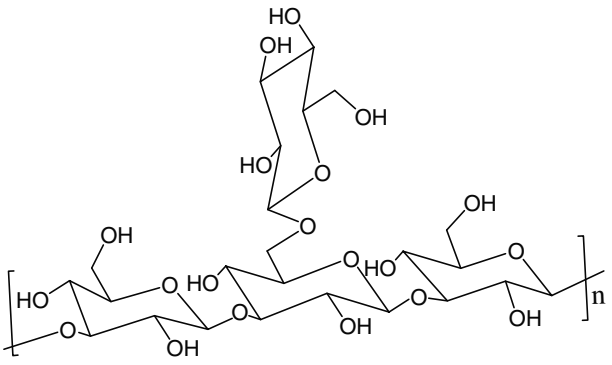

Figure 2. The proposed structure for the native polysaccharide, HEP3.