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Structural characterization of bioactive heteropolysaccharides from the medicinal fungus *Inonotus obliquus* (Chaga).

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Highlights

- Complex heteropolysaccharides were isolated from *Inonotus obliquus* sclerotia.
- The main structural motif in most fractions consisted of (1→3)- and (1→6)-β-Glc.
- A (1→6)-linked α-3-O-Me-Gal was found in *I. obliquus* for the first time.
- The polysaccharides displayed *in vitro* immunomodulatory effects.

Abstract

The aim of this paper was to perform a comprehensive characterization of polysaccharides isolated from the interior (IOI) and exterior (IOE) parts of the fungus *Inonotus obliquus*. Pre-extraction with DCM and MeOH, followed by water and alkali extraction and ethanol precipitation gave two water extracts and two alkali extracts. Neutral and acidic polysaccharide fractions were obtained after anion-exchange chromatography of the water extracts. The neutral polysaccharides (60-73 kDa) were heterogeneous and branched and consisted of a (1→3)-linked β-Glc backbone with (1→6)-linked kinks in the chain at approximately every fifth residue, with branches of (1→6)-linked β-Glc in addition to substantial amounts of (1→6)-linked α-Gal with 3-O-methylation at about every third Gal residue. The acidic polysaccharide fractions (10-31 kDa) showed similar structural motifs as the neutral fractions differing mainly by the presence of (1→4)-linked α-GalA and α-GlcA. β-Xyl, α-Man and α-Rha were also present in varying amounts in all fractions. No major structural differences between the IOI and IOE fractions were observed. An alkaline polysaccharide fraction (>450 kDa) was obtained from the IOI alkali extract, and consisted mainly of (1→3)- and (1→6)-linked β-Glc and (1→4)-linked β-Xyl. Several of the fractions showed *in vitro* immunomodulatory effect by increasing NO production in the murine macrophage and dendritic cell lines J774.A1 and D2SC/1. Most fractions managed to increase NO production only at the highest concentration tested (100 µg/ml), while the neutral

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