DEPOLYMERIZATION OF FUCOSYLATED CHONDROITIN SULFATE FROM SEA CUCUMBER USING HYDROGEN PEROXIDE AND COPPER IONS

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INTRODUCTION: Fucosylated chondroitin sulfate (FCS) is a glycosaminoglycan obtained from sea cucumber consisting of a backbone like that of mammalian chondroitin sulfate: (4-β-D-GlcA-1→3-β-D-GalNAc)n, with branches of sulfated α-fucopyranose (Fucp), substituted at the 3-position of the β-D-GlcA residues. FCS has various biological effects such as anticoagulant, antithrombotic and anti-inflammatory activities due to its unusual branched structure. In order to further evaluate the structure versus activity of FCS, it is important to obtain low molecular weight derivatives from the polysaccharide. OBJECTIVE: This study analyzed sulfated oligosaccharides, obtained by depolymerization with H2O2 in the presence of Cu²⁺, from FCS isolated from L. grisea. MATERIALS AND METHODS: FCS was extracted from the body wall of the sea cucumber L. grisea by papain digestion (60°C/24h), and purified by selective precipitation with ethanol. Depolymerization of FCS by Cu²⁺ at pH 7.0 was obtained through controlled flow of 2%(v/v) H2O2 in a bath at 60 °C during 30, 60 and 90 min. Then, the excess of Cu²⁺ was removed by a strong acidic cationic exchanger Dowex®. The depolymerization process was checked by a 6% polyacrylamide gel electrophoresis (PAGE); thereafter, the oligomers were separated by an exclusion chromatography on a Bio Gel P-10 resin. The purity and integrity of the oligomers were confirmed by NMR spectroscopy. RESULTS: Chemical hydrolysis at different times gradually reduced the molecular size of FCS as indicated by PAGE and gel permeation chromatography. The sulfated oligosaccharides produced after 90min were separated into I-VII fractions and their 1H 1D NMR spectra showed anomic signals at 5.44 and 5.21 of α-fucp, two signals at 3.58 and 3.52 for H3 and H2 β-D-GlcA residues, respectively, and two signals at 1.99 and 1.85 for two β-D-GalNAc. CONCLUSIONS: The approach showed an appropriate method to obtain sulfated oligosaccharides from FCS with well-defined structure. Acknowledgements: CNPq/FAPERJ. Key words: depolymerization, oligosaccharides, sea cucumber.