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Characteristic oligosaccharides released from acid hydrolysis for the structural analysis of chondroitin sulfate

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ABSTRACT

Because the glycosidic linkage of uronic acid is most resistant to acid, oligosaccharides may be formed during the acid hydrolysis of acidic polysaccharides. To take chondroitin sulfate (CS) as an example of acidic polysaccharides, the present study characterized the oligosaccharides released through acid hydrolysis and demonstrated their usefulness for structural confirmation. Acid hydrolysates of commercial standard CSs from shark cartilage and porcine bone were elucidated using HPLC-MS^n after 1-phenyl-3-methyl-5-pyrazolone (PMP) derivatization, and altogether 11 di-, tri- and tetra-saccharides with or without sulfate/acetyl groups were identified by their multi-stage mass spectra. Meanwhile the trends of reaction yields of these oligosaccharides alone with trifluoroacetic acid (TFA) concentrations (0.1–2.0 M) were investigated, and 0.2 M TFA was recommended. Then three real samples, sturgeon backbone, porcine trachea and sea cucumber were analyzed, and their CSs were identified by detection of characteristic oligosaccharide fragments. The present study indicated that acid hydrolysis could provide information for acetyl substitution, sulfation and glycosidic linkages, and was helpful for the structural analysis of acidic polysaccharides.

1. Introduction

Chondroitin sulfate (CS) is a kind of glycosaminoglycan (GAG), which serves multiple important biological roles as side chains of proteoglycans in vertebrates and invertebrates [1–4]. Moreover, CS has been widely used in pharmaceutical and nutraceutical industry due to its favorable bioactivities, such as promoting bone formation, reducing blood lipids, and inhibiting tumor cells and viruses [5–8]. Acid hydrolysis is economical and simple for polysaccharide depolymerization, and it is commonly applied in monosaccharide composition analysis of polysaccharides [9–12]. The monosaccharide ratios of polysaccharides are determined based on monosaccharides released from polysaccharides [13]. However, for acidic polysaccharides, such as chondroitin sulfate, acid hydrolysis could not accurately evaluate the monosaccharide composition, because uronic acids are unstable in acidic solution [14], and moreover, the glycosidic linkage of uronic acid is extremely difficult to cleave by acid. Whereas, acid hydrolysis is still helpful for the structural analysis of acidic polysaccharides. Due to the most resistance of glycosidic linkage of uronic acid, oligosaccharides may be formed during the acid hydrolysis of acidic polysaccharides [15]. In our previous studies, disaccharides with uronic acid at the non-reducing end were found in the acid hydrolysates of acidic polysaccharides, and they gave hints for identification of these polysaccharides [16,17]. However, disaccharides alone could not provide the information about the linkages between disaccharide units, and more oligosaccharides are needed to confirm the polysaccharide structures.

Thus, the present study aimed to reveal the oligosaccharides released from acidic polysaccharides through acid hydrolysis with different acid concentration, which could provide more information on the CS structures and demonstrate their usefulness for structural elucidation. Moreover, since acid hydrolysis is an

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universal method to depolymerize polysaccharide, our demonstrated method could analyze substituted CS, such as fucosylated CS in sea cucumber, which could not be cleaved by enzymatic digestion such as chondroitin ABC lyase due to their fucose branches at C-3 of GlcA residues [18–20]. In the present study, CS, which consists of alternating disaccharide units of →4GlcAβ1→3GalNAcβ1→, was chosen as a representative of acidic polysaccharides. Acid hydrolysates of 2 commercial standard CSs were analyzed to find characteristic oligosaccharides, and then these oligosaccharides were used as markers to characterize CSs in three biological samples, sturgeon backbone, porcine trachea and sea cucumber.

2. Materials and methods

2.1. Materials

Trifluoroacetic acid (TFA) and ammonium acetate were obtained from Aladdin Industrial Corporation (Shanghai, China). Chondroitin sulfate C sodium salt from shark cartilage was purchased from Sigma-Aldrich (Germany). Chondroitin sulfate sodium from porcine bone was from J&K Scientific (Beijing, China). PMP was acquired from Sinopharm Chemical Reagent Co. (Beijing, China). Porcine trachea was collected from supermarket in Dalian, China. Sturgeon backbone (*Acipenser gueldenstaedti*) was acquired from fish farm of Quzhou Sturgeon Science & Technology Development Co., Ltd. Sea cucumbers (*Stichopus japonicus*) were purchased from a local market in Dalian, China.

2.2. Isolation of polysaccharides from sturgeon backbone, porcine trachea and sea cucumber

Sturgeon backbone and porcine trachea were cleaned, crushed and then freeze-dried, respectively. 1.0 g of dry sample was digested in 25 mL of PBS buffer (0.05 M, pH 8.0) containing Cys-EDTA-Na_2 (0.05 M) and trypsin (5%, w/w), and was incubated at 37 °C for 4 h. Later, papain (5%, w/w) was added and the solution was further incubated at 65 °C for 3 h. Proteases were inactivated by heating at 100 °C for 5 min. Finally, ethanol (37.5 mL) was added to the solution and the precipitate was collected and lyophilized for further use. Polysaccharides of crude sea cucumbers were prepared according to the method reported previously [21].

2.3. Acid hydrolysis

Commercial standard CSs from shark cartilage and from porcine bone were individually dissolved in a series of TFA concentrations of 0.1, 0.2, 0.5, 0.8, 1.1, 1.4, 1.7, and 2.0 M to achieve a CS concentration of 1.5 mg/mL, and heated in a sealed tube at 100 °C for 1 h. In another set, 20 mg crude polysaccharides from porcine trachea, sturgeon backbone and sea cucumber were hydrolyzed with 1.0 mL of 0.2 M TFA at 100 °C for 1 h separately. The reaction solutions were all dried using vacuum freeze dryer (Labogene ScanSpeed, SCANVAC Coolsafe). Then 0.5 mL of distilled water was added and concentration by centrifugation was repeated until TFA was fully vaporized.

2.4. 1-phenyl-3-methyl-5-pyrazolone (PMP) derivatization

The sample was dissolved in 400 μL of 25% ammonia and mixed with 400 μL of 0.3 M PMP methanolic solution in a fume hood. After the reaction mixture was cooled to room temperature, it was added 1 mL of methanol and dried using a centrifugal concentrator, and repeated twice until the NH_3 was fully evaporated. Subsequently, water and chloroform (1.0 mL each) were added, and the mixture was shaken vigorously. The chloroform layer was discarded, and the extraction process was repeated three times. Then the aqueous layer was filtered through a 0.22 μm pore membrane filter for the HPLC-MS^n analysis.

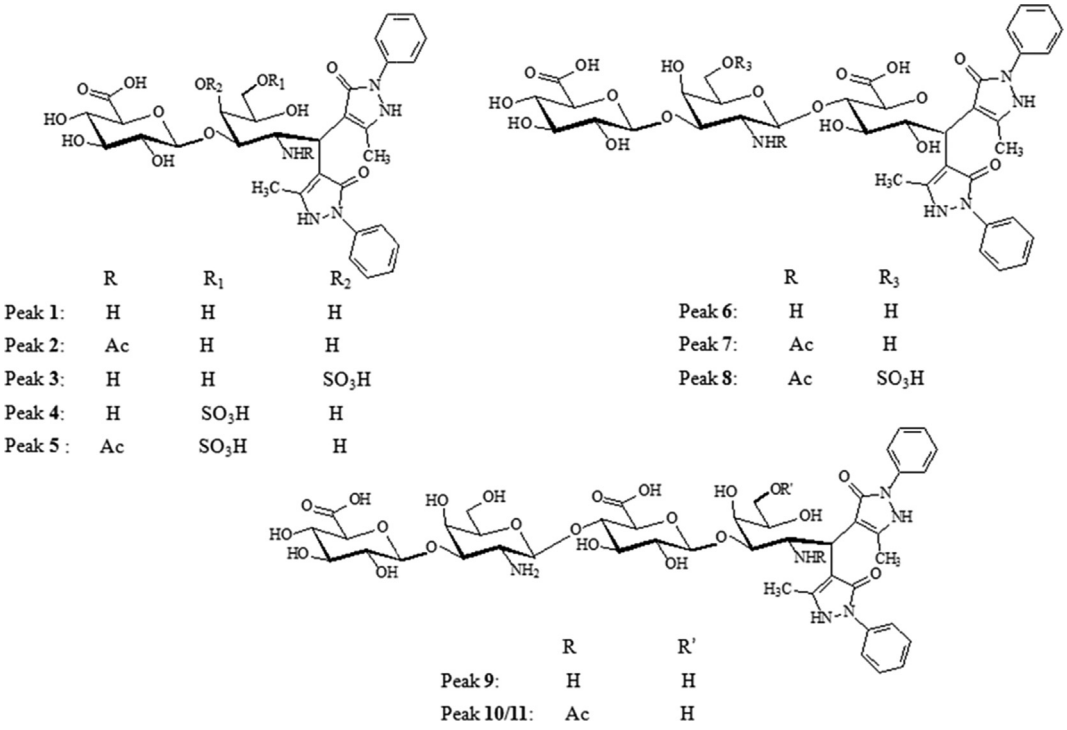

Fig. 1. Structures of PMP-labeled oligosaccharides derived from CSs.