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REGULAR ARTICLE

Polysaccharides from the skin of the ray *Raja radula*. Partial characterization and anticoagulant activity

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Abstract

Introduction: The polysaccharide fraction from the skin of the ray *Raja radula* was extracted, characterized and assayed for anticoagulant activity.
Materials and methods: A whole polysaccharidic fraction was extracted from the skin of the ray *Raja radula* by papain digestion followed by cetylpyridinium chloride and ethanol precipitation and was subjected to gel chromatography and anion exchange chromatography, acetate cellulose electrophoresis and characterized by physicochemical procedures. APTT and anti Xa assays were performed to assess the anticoagulant activity of the polysaccharidic fractions in comparison with unfractionated heparin.
Results: Gel and anion-exchange chromatography revealed two negatively charged polysaccharidic populations different in both molecular weight and charge. Infrared spectra suggested the occurrence of uronic acids and acetylated hexosamines. The second polysaccharide was highly sulfated, with a sulfate content of approximately 29%. These data suggested that dermatan sulfate (DS) is the sulfate rich polysaccharide whereas hyaluronic acid (HA) is the polysaccharide devoid of sulfate groups. Molecular mass characterization indicated that their average molecular masses were 22 kDa and 85 kDa, respectively. The sulfated polysaccharide, i.e. presumably DS, accounted alone for the observed concentration-dependent anticoagulant activity which was, as measured by APTT, 2 to 3-fold lower than that of heparin. In addition, it had a significant anti-Xa activity.

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Conclusion: A major-sulfated polysaccharide, likely a dermatan sulfate, was extracted from the ray Raja radula skin. The results indicated that it exhibited a high anticoagulant activity and suggested that it was mediated by both heparin cofactor II and antithrombin.
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Glycosaminoglycans are widely distributed in a variety of tissues from different biological origins. Their structure consists of a repeated disaccharide unit containing uronic acid and hexosamine residues, which may bear different chemical substituents, mainly sulfate and N-acetyl groups. These polysaccharides possess a variety of biological activities such as the well described anticoagulant activity for the sulfated glycosaminoglycans [1].

Glycosaminoglycans can be extracted by various procedures such as sodium dodecyl sulfate [2], enzyme digestion, cetylpyridinium chloride and ethanol precipitation [3,4]. Purification is mainly carried out by gel filtration [5] or by anion exchange chromatography. Structure analysis is made by chemical and physical methods including polyacrylamide and agarose gel electrophoresis, infrared and NMR spectroscopy [4,6].

Sulfated glycosaminoglycans exert their anticoagulant effect through interactions with the serine-proteinases of the coagulation system and their physiological inhibitors antithrombin (AT) and heparin cofactor II (HCII) [1].

Among glycosaminoglycans endowed with anticoagulant activity, heparin and dermatan sulfate bind to AT and /or HCII via a specific high affinity AT-binding pentasaccharide for heparin [7–9] and a high affinity HCII-binding hexasacharide for dermatan sulfate [10,11].

In vivo, although heparan sulfate tends to be emphasised as the most biologically active glycosaminoglycan [12,13], dermatan sulfate is of particular interest due to its expression in numerous mammalian tissues and its predominance in skin. Dermatan and dermatan sulfate proteoglycans involvement have also been evidenced in cardiovascular diseases, tumorigenesis, infection, wound repair, and fibrosis [14]. Moreover dermatan sulfate was demonstrated as an important cofactor in a variety of cell behaviours [14].

Glycosaminoglycans such as heparin and dermatan sulfate are usually extracted from mammalian tissues. Some have recently been isolated from marine invertebrates [15]. Marine origin may constitute a safer alternative with no infectious risk due to viruses or to prions. In this study, we extracted a whole polysaccharidic fraction from the ray skin of *Raja radula*. Data suggested it contains hyaluronic acid (HA) and a sulfate-rich dermatan sulfate-like fraction endowed with anticoagulant activity.

Materials and methods

Materials

Ray (*Raja radula*) skin was purchased from market, Tunisia. Unfractionated heparin (peak 15 kD) was from LEO, Panpharma SA, France, chondroitin sulfate from bovine trachea and hyaluronic acid were from Fluka, Germany, galactose from Park Scientific Ltd. USA, Sephadex G100-120 from Sigma, Sweden, papain (3U/mg) from Merck, Darmstadt, Germany, Quantichrom™ Sulfate Assay Kit containing barium chloride and polyethylene glycol 6000 (reagent A) and trichloroacetic acid (reagent B) was from BioAssay Systems, USA, PTT AUTOMATE containing cephalin from rabbit cerebral tissues and a particulate activator (silica), and Staclot® Heparin containing substrate plasma of human origin (Reagent 1), bovine factor Xa 0.3 UI/mL (Reagent 2) and phospholipids in calcium containing medium (35 mM) (Reagent 3) were from Diagnostica Stago, Asnières, France, Owren-Koller buffer was from Biomaghreb, Tunisia, platelet poor plasma (PPP) was prepared from whole blood, drawn on sodium citrate (9:1, v/v), obtained by venipuncture of healthy volunteers.

Methods

Polysaccharide extraction

Ray skin was sun-dried, cut into small pieces and grinded with a mechanical crusher. The extraction of the crude polysaccharide fraction was carried out according to the following procedure. 5 g alcohol insoluble substances (AIS) are dissolved in 250 mL sodium acetate 0.1 M, EDTA 5 mM, cystein 5 mM pH6. 510 mg papain are added, and the mixture left 24 hours at 60 °C. The mixture is then left to cool down at room temperature and filtered. The residue is washed with 138 ml distilled water and filtered again. The filtrates are mixed and polysaccharides are precipitated with 20 mL cetylpyridinium chloride 10% (w/v). The mixture is left 24 hours at room temperature and centrifuged 30 min at 5000 tr/min at 4 °C. The pellet is washed with 610 mL cetylpyridinium chloride 0.05% (w/v), and then dissolved in 172 mL NaCl solution in ethanol (100:15, v/v). 650 mL ethanol are added. The polysaccharide containing solution is left 24 hours at 4 °C then centrifuged 30 min at 5000 tr/min at 4 °C. The pellet is washed twice with ethanol 80% then once with absolute ethanol. The pellet is then redissolved in desionised water and lyophilised [16,17]. It is dialysed against desionized water to provide the crude polysaccharide fraction termed RED80.

Gel filtration

Gel filtration was carried out in sodium acetate 0.05 M pH6: 2 ml polysaccharide 1 mg/ml are applied on a sephadex G-100 column (2.5×70 cm) at a flow rate 10 ml/h. 2.5 ml fractions are collected (5). UV detection was at 215 nm. A calibration curve was performed with low molecular weight heparin (LMWH) (peak 5 kDa) and unfractionated heparin (peak 15 kDa) from LEO, Panpharma SA, France, and dextrans (40 kDa and 70 kDa) from Sigma, Germany.

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