Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Research 342 (2007) 2326–2330
Carbohydrate RESEARCH

Note

A sulfated fucan from the brown alga *Laminaria cichorioides* has mainly heparin cofactor II-dependent anticoagulant activity

Seon-Joo Yoon,[a,b,†] Yu-Ryang Pyun,[b] Jae-Kwan Hwang[b] and Paulo A. S. Mourão[a,*]

[a]*Laboratório de Tecido Conjuntivo, Hospital Universitário Clementino Fraga Filho and Programa de Glicobiologia, Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro, Caixa Postal 68041, Rio de Janeiro, RJ 21941-590, Brazil*
[b]*Department of Biotechnology, and Bioproducts Research Center, Yonsei University, Seoul 120-749, Republic of Korea*

Received 23 January 2007; received in revised form 5 June 2007; accepted 18 June 2007
Available online 28 June 2007

Abstract—The major acidic polysaccharide from the brown alga *Laminaria cichorioides* is a complex and heterogeneous sulfated fucan. Its preponderant structure is a 2,3-disulfated, 4-linked α-fucose unit. The purified polysaccharide has a potent anticoagulant activity, as estimated by APTT assay (~40 IU/mg), which is mainly mediated by thrombin inhibition by heparin cofactor II. It also accelerates thrombin and factor Xa inhibition by antithrombin but at a lower potency. Sulfated fucan from *L. cichorioides* is a promising anticoagulant polysaccharide and a possible alternative for an antithrombotic compound due to its preferential heparin cofactor II-dependent activity.

Keywords: Anticoagulant; Heparin cofactor II; Sulfated polysaccharide; Brown alga; *Laminaria cichorioides*

Sulfated fucans are water-soluble polysaccharides that occur as the major constituent of brown algae. They are obtained in large quantities and are among the most abundant sulfated polysaccharides found in nature.[1] More recently, sulfated fucans were also found in several tissues of marine invertebrates. These polysaccharides have a wide variety of biological properties but the anticoagulant activity is by far the most well studied.[1] Studies with the invertebrate polysaccharides revealed some structure–anticoagulant activity relationship concerning the sulfated fucans.[1,2]

The structures of algal sulfated fucans are complex and heterogeneous. They also vary among species.[1,3] Studies using a sulfated fucan from the brown alga *Fucus vesiculosus* suggested that the antithrombin activity is mediated mainly by heparin cofactor II, with a minor contribution of antithrombin.[4] In contrast, a sulfated fucan from the alga *Ascophyllum nodosum* has an intense antithrombin-mediated anticoagulant activity.[5] Possibly, sulfated fucans from different species may vary in the mechanism of their anticoagulant activity because of their different chemical structure.[6,7] However, it is difficult to compare these results since the anticoagulant assays were performed in different laboratories using distinct protocols, and sulfated fucans were purified using a variety of methodologies.

In the present study, we investigated the structure and anticoagulant activity of a sulfated polysaccharide extracted from the brown alga *Laminaria cichorioides*. The crude acidic polysaccharide showed increasing clotting times in the APTT and TT assays that were proportional to its concentration. No change in the PT clotting time was observed (Table 1). We purified the polysaccharide using an anion-exchange chromatography on Mono-Q (Fig. 1a). The major subfraction (S2) showed strong metachromatic property, high content of sugar and low content of hexuronic acid. Subfraction S1 showed a broad peak, eluted at low salt concentration while subfraction S3 was eluted at high NaCl concentration.

Abbreviations: APTT, activated partial thromboplastin time; PT, prothrombin time; TT, thrombin time

* Corresponding author. Tel./fax: +55 21 2562 2090; e-mail: pmourao@hucff.ufrj.br
† Present address: Pacific Northwest Research Institute, and University of Washington, Seattle, WA 98122, USA.

0008-6215/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2007.06.019

Table 1. Concentration-dependent anticoagulant activity of crude acidic polysaccharides from *L. cichorioides*

Concentration[a] (ug/mL)	Clotting time[b] (s)		
	APTT	TT	PT
0	40 ± 0.7	10 ± 0.9	14 ± 1.0
10	55 ± 1.1	115 ± 5.8	14 ± 1.0
30	112 ± 2.8	>600	17 ± 2.1
50	231 ± 2.1	>600	23 ± 3.5

[a] The lyophilized crude polysaccharide was dissolved in human platelet-poor plasma (hPPP) to each concentration shown.
[b] Each clotting time represents the average of triplicate experiments using hPPP. Data are shown as means ± SD in s.

Agarose gel electrophoresis showed differences in mobility among the purified subfractions (Fig. 1b). Each subfraction showed very clear bands. S1 and S2 showed a single, homogeneous and almost coincident metachromatic band, while S3 showed an addition second band with higher mobility. The average molecular sizes of the three subfractions were estimated by polyacrylamide gel electrophoresis (Fig. 1c). The unfractionated polysaccharides showed a wide dispersion in their molecular sizes. Subfractions S1 and S2 showed a similar mobility as standard dextran sulfate (~8 kDa) and chondroitin 6-sulfate (~60 kDa), respectively, whereas subfraction S3 remained close to the origin of the gel, denoting a high molecular mass. Gel filtration chromatography on Sephacryl S-400 confirmed the average molecular mass of subfraction S2 as ~60 kDa (not shown).

Chemical analysis (Table 2) revealed that fucose is the major sugar component of subfraction S2 while galactose and glucose prevail in subfractions S1 and S3, respectively. Subfraction S2 has also high sulfate content. Approximately two sulfate esters are present per sugar unit.

Subfraction S2 is the preponderant anticoagulant polysaccharide in the water extract of this alga, with an anticoagulant activity of 40 IU/mg when compared with a heparin standard (193 IU/mg), as determined by the APTT assay (not shown). The crude water extract obtained from this alga has an anticoagulant activity of 25 IU/mg. The anticoagulant activity of the three subfractions has no correlation with their molecular size.

We attempted to determine the major structural component of the sulfated fucan from *L. cichorioides* using NMR and methylation analysis. The assignment of the major peaks was achieved by analysis of ^{1}H one-dimensional NMR, ^{1}H COSY, ^{1}H TOCSY, and $^{1}H/^{13}C$ HMQC spectra. The spectra of subfraction S2 showed broader and poorly resolved signals indicating a clearly heterogeneous chemical structure (data not shown). However, peaks clearly attributable to α-anomeric protons were identified in the ^{1}H NMR and $^{1}H/^{13}C$ HMQC spectra. The signals in the vicinity of 5.5 ppm of the ^{1}H NMR spectra showed the preponderance of α-anomeric protons. Comparison between chemical shifts observed

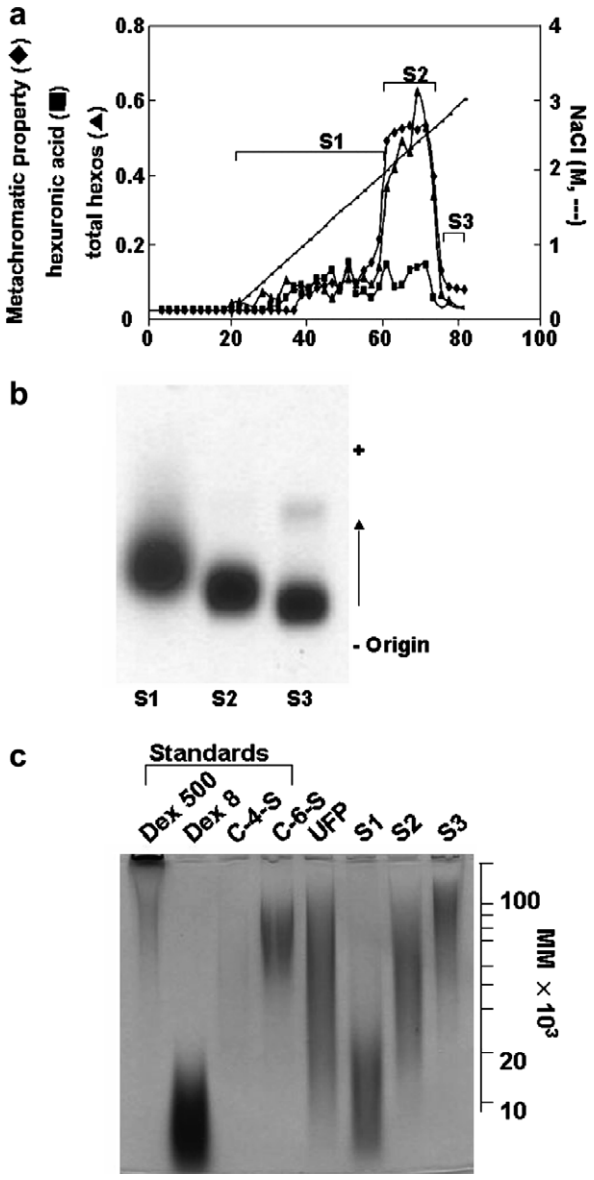

Figure 1. Purification of the sulfated polysaccharides from the brown alga *L. cichorioides* by anion-exchange chromatography (a), analysis of the purified fractions by agarose gel electrophoresis (b) and estimation of their average molecular masses by polyacrylamide gel electrophoresis (c). (a) Acidic polysaccharides (~60 mg) obtained from the brown alga *L. cichorioides* were applied to Mono Q-FPLC column and purified as described under 'Experimental'. Fractions were checked by the phenol–H_2SO_4 (▲) and carbazole (■) reactions, for metachromasia (◆) and NaCl concentration (---). The fractions were pooled into three subfractions, denominated as S1, S2, and S3 (see horizontal bars in the panel). The purified subfractions (~15 μg) were analyzed by agarose gel electrophoresis (b) and by polyacrylamide electrophoresis (c). For this last experiment, the molecular markers used were low-molecular-weight dextran sulfate (Dex 8) (8 kDa), chondroitin 4-sulfate from whale cartilage (C-4-S) (40 kDa), chondroitin 6-sulfate from shark cartilage (C-6-S) (60 kDa) and high-molecular-weight dextran sulfate (Dex 500) (~500 kDa). UFP, unfractionated polysaccharide.

for the preponderant residue of subfraction S2 with the literature data indicates that this polysaccharide