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Preparation, structure and anticoagulant activity of a low molecular weight fraction produced by mild acid hydrolysis of sulfated rhamnan from *Monostroma latissimum*

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ABSTRACT

A low molecular weight fraction, designated LMWP, was prepared by mild acid hydrolysis of sulfated rhamnan from *Monostroma latissimum* and purified by anion-exchange and gel-permeation chromatography. Chemical and spectroscopic analyses showed that LMWP was mainly composed of rhamnose, and its molecular weight was about 33.6 kDa. The backbone of LMWP consists of 1,3-linked α-L-rhamnose units with partially sulfate groups at the C-2 position. Approximately 25% of 1,3-linked α-L-rhamnose units is substituted at C-2 by sulfated or non-sulfated 1,3-linked α-L-rhamnose and 1,2-linked α-L-rhamnose units. LMWP effectively prolonged clotting time as evaluated by the activated partial thromboplastin time assay and was a potent thrombin inhibitor mediated by heparin cofactor II. The investigation demonstrated that LMWP is a novel sulfated polysaccharide with anticoagulant activity.

1. Introduction

Algae often produce sulfated polysaccharides with unique structures and functions (Hayashi et al., 2008; Matsubara et al., 2001). Some polysaccharides from green algae have good anticoagulant activities, especially sulfated polysaccharides from *Monostromacceae* species (Hayakawa et al., 2000; Lee et al., 1998; Mao et al., 2009). Because of their high molecular weights (200–870 kDa), the absorptivity and bioavaibabity of the sulfated polysaccharides as a possible food supplement is decreased (Harada and Maeda, 1998; Li et al., 2011; Zhang et al., 2008).

Partial depolymerization of native polysaccharides is possible (Linhardt et al., 1994), and Nardella et al. (1996) reported that the anticoagulant activities of low molecular weight fucan obtained by degradation of native fucan from *Ascophyllum nodosum* were similar as evaluated by activated partial thromboplastin time assays. A low molecular weight sulfated galactan obtained by hydrogen peroxide degradation of sulfated galactan from *Schizymenia binderi* had slightly lower anticoagulant activity than the native polysaccharide (Zúñiga et al., 2006). Zhang et al. (2008) reported that the low molecular weight fractions (26–216 kDa) prepared by hydrogen peroxide degradation of sulfated polysaccharide from *Monostroma latissimum* (a wild alga) had higher anticoagulant activities than the parent polysaccharide. The low molecular weight fractions F-I and F-II from sulfated galactan of *Botryocladia occidentalis* had high anticoagulant activities, and total thrombin inhibition of F-I and F-II in the presence of heparin cofactor II was even stronger than that of the intact polysaccharide (Melo et al., 2004). The low molecular weight fractions prepared by partial depolymerization of native polysaccharide from algae represent a prospective source of anticoagulant to be explored. In this study, a low molecular weight fraction was prepared by controlled acid hydrolysis of sulfated rhamnan from *M. latissimum* (a cultured alga), and its structure and anticoagulant activity were investigated.

2. Methods

2.1. Materials

Sulfated rhamnan was from *M. latissimum* (a cultured alga). Activated partial thromboplastin time (APTT) assay reagent was from Shanghai Sun (Shanghai, China). Thrombin, heparin cofactor II, antithrombin and chromogenic substrate S-2238 were from Chromogenix AB (Mondal, Sweden). Q Sepharose Fast Flow and Sephadex G150 were from Pharmacia Bioscience (Uppsala, Sweden). Dialysis membranes (molecular weight cut off 3500)

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