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# Marine chondroitin sulfate of defined molecular weight by enzymatic depolymerization

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ABSTRACT

Chondroitin sulfate (CS) is a sulfated glycosaminoglycan with diverse biological activities, which are influenced by molecular weight (Mw) and sulfation pattern. In the present work, we take advantage of the characteristic high Mw of fish CS (51–70 kDa) to obtain lower Mw fragments with hyaluronidase and chondroitinase ABC. With this aim, we present a pseudo-mechanistic model capable of reproducing the decrease in Mw of CS from five different fish species over 24 h at four enzyme to substrate ratios. The fitting parameters of the model for each species allow to establish conditions of reaction to produce CS of the desired Mw. Furthermore, the main features of the sulfation pattern of fish CS remain in the depolymerized fragments, highlighting the feasibility of the proposed approach.

## 1. Introduction

Chondroitin sulfate (CS) is a sulfated polysaccharide of the glycosaminoglycan family widely distributed at the cell surface and in the extracellular matrix of most animal tissues, where it participates in fundamental cellular events such as cell communication, differentiation and growth (Yamada & Sugahara, 2008). These biological properties make CS interesting for several applications such as cartilage regeneration, either as a nutraceutical or incorporated into tissue engineering scaffolds, nerve regeneration, and as an anticoagulant, anti-inflammatory and anti-metastatic agent (Valcarcel, Novoa-Carballal, Pérez-Martín, Reis, & Vázquez, 2017).

Bioactivity of CS depends largely on its capacity to interact with proteins, which is determined by CS chemical properties and related structure (Benito-Arenas et al., 2018; Djerbal, Lortat-Jacob, & Kwok, 2017; Miller & Hsieh-Wilson, 2015). CS consists of a sequence of glucuronic acid (GlcA) and *N*-acetyl galactosamine (GalNAc) linked by alternating β-(1→4) and β-(1→3) glycosidic bonds. Sulfation can occur at different positions in both rings, giving rise to distinct units sequentially arranged in polymeric chains of variable length. As a result, considerable heterogeneity exists in CS in terms of charge density, sulfation pattern and molecular weight (Kjellén & Lindahl, 2018; Valcarcel et al., 2017).

Marine CS differs from terrestrial counterparts both in terms of sulfation and molecular weight. The latter contains a majority of 4-sulfated GalNAc units (CS-A), while in marine animals 6-sulfated GalNAc (CS-C) generally prevails, accompanied by a significant proportion of disulfated disaccharides (López-Álvarez et al., 2019). Furthermore, diversity in sulfation is larger in the marine environment, discovering in some cases unique units and structures, *e.g.* CS-K (GlcA3S-GalNAc4S) in octopus with neuritogenic activity (Higashi et al., 2015) and fucosylated CS in sea cucumbers with anticoagulant activity (Wu et al., 2012).

Beyond sulfation, molecular weight also appears as an important parameter of CS bioactivity. Low molecular weight CS has shown potential for attenuation of ostheoarthritis by inhibiting the complement system, especially depolymerized shark CS (1.5 kDa) (Li et al., 2016). Also, the permeability of CS seems to increase as molecular weight decreases, as shown for dietary supplements tested in an *in vitro* intestinal model (Adebowale, Cox, Liang, & Eddington, 2000). However, low molecular weight CS clears from the blood more rapidly than intact CS, as indicated by the positive correlation found between molecular weight (6–50 kDa) and the plasma half-life of CS intravenously injected to mice (Sakai et al., 2002). As a biomaterial, the cellular uptake and transfection efficiency of complexes of CS with chitosan and plasmid DNA for gene delivery have shown to depend on CS molecular weight, with CS of 22 kDa showing the best results (Hagiwara, Nakata, Koyama, & Sato, 2012). Therefore, CS molecular weight presents as an essential parameter to control depending on the application.

Furthermore, while acting on sulfation is challenging and is mostly

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