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Synthesis, physicochemical characterization and biological evaluation of chitosan sulfate as heparan sulfate mimics

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ABSTRACT

Despite the relevant biological functions of heparan sulfate (HS) glycosaminoglycans, their limited availability and the chemical heterogeneity from natural sources hamper their use for biomedical applications. Chitosan sulfates (ChS) exhibit structural similarity to HSs and may mimic their biological functions. We prepared a variety of ChS with different degree of sulfation to evaluate their ability to mimic HS in protein binding and to promote neural cell division and differentiation. The structure of the products was characterized using various spectroscopic and analytical methods. The study of their interaction with different growth factors showed that ChS bound to the proteins similarly or even better than heparin. In cell cultures, a transition effect on cell number was observed as a function of ChS concentration. Differences in promoting the expression of the differentiation markers were also found depending on the degree of sulfation and modification in the chitosan.

1. Introduction

Heparan sulfate proteoglycan (HSPG) represents a group of glycoproteins that are present in the extracellular matrix (ECM) or on the cell surface of essentially all animal cells. HSPGs have structural functions and are involved in numerous biological processes (Lindahl & Li, 2009; Sarrazin, Lamanna, & Esko, 2011). They contribute to the structural integrity of ECM through binding with other ECM components, such as collagen I and IV, fibronectin and laminin. HSPG in the ECM can also bind cytokines, chemokines, growth factors, and morphogens, creating a protecting depot of these proteins that are released by selective degradation of the heparan sulfate chains. One of the most relevant aspects of HSPGs located in the ECM or inserted in the cell membrane is that they can act as co-receptors. In this role, they serve as templates that facilitates the binding of growth factors to their cell surface receptor (Xu & Esko, 2014). As a result of these interactions, HSPGs modulate diverse processes such as angiogenesis, cell differentiation, growth and migration.

HSPGs consist of a core protein covalently linked to one or several polysaccharide chains of heparan sulfate (HS). HS is a type of glycosaminoglycan composed of repeating units of D-glucuronic (GlcA) or L-iduronic (IdoA) acid linked to 2-amino-2-deoxy D-glucopyranose (GlcN) (Fig. 1a). These disaccharide units may be sulfated at C3 and C6 of GlcN and at C2 of the uronic acid, and the GlcN amine function may be sulfated, acetylated or unsubstituted. The ability of HSPGs to bind protein ligands is in large part a function of their HS moieties. The highly sulfated chain of HS binds to proteins mainly through electrostatic interactions between their sulfate groups and positively charged groups on the surface of the protein. The specificity and the affinity of a protein for HS chains depends largely on the sulfation profile and chain length of HS (Capila & Linhardt, 2002).

The wide range of interactions of HS, which are relevant to many disease processes, has converted these biomolecules into novel targets for drug development (Gandhi & Mancera, 2010; Weiss, Esko, & Tor, 2017). An important problem, however, for this purpose is the limited availability of homogeneous HS with well-defined sulfation profiles. Chitosan is a natural polysaccharide composed of randomly distributed β-(1 → 4) linked GlcN and GlcNAc (Fig. 1b).Chitosan sulfates (ChS) present structural analogy with heparin and heparan sulfates and consequently may mimic their biological functions. As a heparin mimics,

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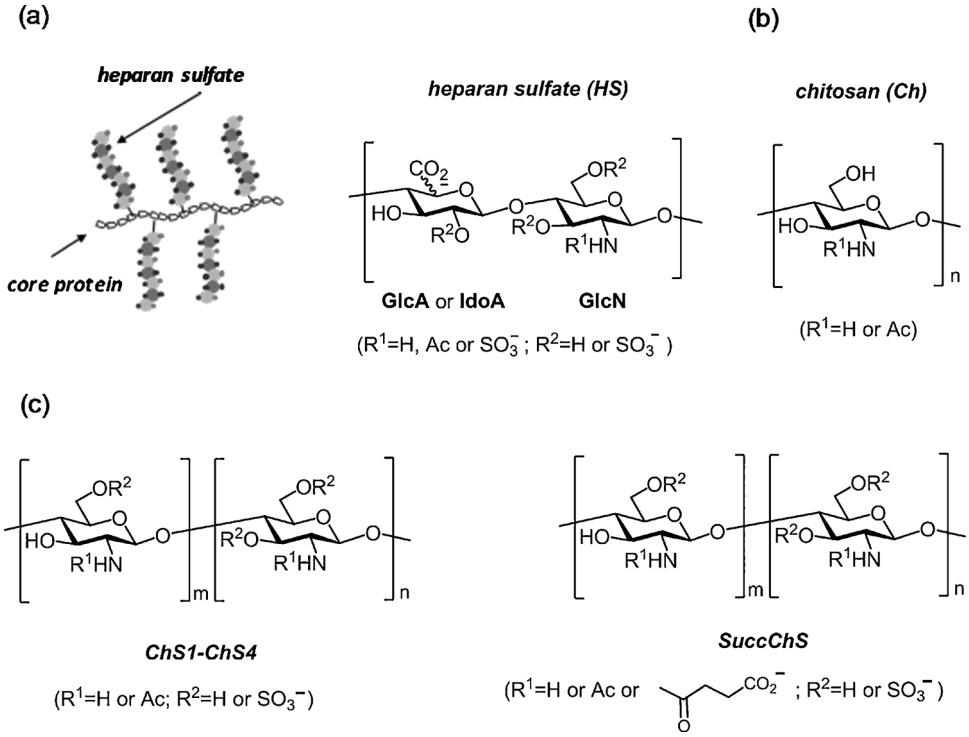

Fig. 1. (a) Schematic representation of the structure of a proteoglycan and of HS. (b) Chemical structure of chitosan (Ch). (c) Chemical structure of chitosan sulfates prepared in this work.

ChS have been shown to display anticoagulant activity (Vikhoreva et al., 2005; Yang et al., 2013). Due to their capacity for binding to protein growth factors, ChS have been used in the preparation of delivery systems for tissue repair and regeneration (Kong, Wang, Cao, Yu, & Liu, 2014). It has been shown that ChS protect growth factors from proteolytic digestion (Li, Ma, Li, & Gao, 2014; Weltrowski et al., 2012). The effects of sulfated chitosan with different sulfation sites on neural differentiation of embryonic stem cells (ESCs) has been reported (Ding et al., 2014). It was found that all the sulfated derivatives can direct neural differentiation to varying degrees, and 6-sulfated chitosans were the most efficient. All these properties, together with the low cytotoxicity of ChS, make them suitable candidates for biomedical applications.

We have recently initiated a project addressed to the preparation of new biomaterials based on chitosan for regeneration of the central nervous system (CNS). This involves the preparation of sulfated chitosans with affinity for growth factors and their use in promoting neural cell differentiation. We hypothesize that the interaction of sulfated chitosans with proteins and the effect on neural cells might depend not only on the degree of sulfation but also on other physicochemical factors. In the present paper we report the synthesis and biological evaluation of several chitosan derivatives with sulfate group at position 6 and, to a lesser extent, at position 3, using chlorosulfonic acid under homogeneous conditions (Fig. 1c) (Zhang et al., 2010). A sulfated succinyl-chitosan, bearing a carboxyl group, has also been synthesized with the aim of both mimicking the gluco/iduronic units of heparan sulfate and enabling the conjugation of the chitosan derivative with other molecules (Fig. 1c). The structure of the chitosan derivatives was characterized by FT-IR, ^{1}H and ^{13}C NMR, GPC, element analysis, UV–vis absorption, circular dichroism, and ζ-potential. The interaction of the chitosan derivatives with several growth factors (GFs) was determined. Finally, the effects of these derivatives on neural precursor cells were evaluated. The biological results are discussed with the information of the sulfation profile, relative surface charges and size of sulfated chitosans obtained from the physical-chemical characterization.

2. Materials and methods

2.1. Materials

Low-molecular-weight chitosan (MW: 50,000–190,000 Da, 85% deacetylated estimated by NMR) was purchased from Sigma-Aldrich. FT-IR spectra were recorded with KBr pellets on a Perkin Elmer Spectrum One spectrophotometer. ^{1}H NMR spectra were registered at 400 or 500 MHz and ^{13}C NMR spectra were obtained at 100 or 125 MHz on Varian INOVA and Varian SYSTEM spectrometers, respectively. Elemental analysis were determined in a Heraus CHN-O analyser.

2.2. Synthesis of sulfate chitosans (SuccCh, ChS1-ChS4, SuccChS)

Sulfated chitosans were synthesized according to previously reported methods (Skorik et al., 2017; Wang et al., 2012; Zhang et al., 2010) with slight modifications. The procedures are described in the Supplementary material.

2.3. Chitosan derivatives characterization

Chitosan viscosity-average molecular weight was 129 kDa as determined by viscometric measurements using an Ubbelohde capillary viscometer type 525/20. The average molecular weight was calculated from Mark–Houwink–Sakurada equation (Eq. (1))

$$[\eta] = KM_v^{\alpha} \quad (1)$$

where [η] is the intrinsic viscosity, Mv the viscosity-average molecular weight, and K are constants for given solute-solvent system and temperature. Chitosan sample was dissolved in 0.2 M Ammonium Acetate and 0.3 M Acetic Acid solution (pH 4.5) and the measurement was carried out at 25 °C. In these conditions K = 0.076 (ml/g) and α = 0.776 when the intrinsic viscosity is expressed in ml/g (Rinaudo, Milas; & Dung, 1993).