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Research review paper

Glycosaminoglycans from marine sources as therapeutic agents

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ABSTRACT

Glycosaminoglycans (GAGs) in marine animals are different to those of terrestrial organisms, mainly in terms of molecular weight and sulfation. The therapeutic properties of GAGs are related to their ability to interact with proteins, which is very much influenced by sulfation position and patterns. Since currently GAGs cannot be chemically synthesized, they are sourced from natural products, with high intra- but also inter-species variability, in terms of chain length, disaccharide composition and sulfation pattern. Consequently, sulfated GAGs are the most interesting molecules in the marine environment and constitute the focus of the present review. In particular, chondroitin sulfate (CS) appears as the most promising compound. CS-E chains [GlcA-GalNAc(4S,6S)] extracted from squid possess antiviral and anti-metastatic activities and seem to impart signalling properties and improve the mechanical performance of cartilage engineering constructs; Squid CS-E and octopus CS-K [GlcA(3*S*)-GalNAc(4S)], dermatan sulfate (DS) from sea squirts [-iK units, IdoA(3S)-GalNAc(4S)] and sea urchins [-iE units, IdoA-GalNAc(4S,6S)] and hybrids CS/DS from sharks (-B/iB [GlcA/IdoA(2S)-GalNAc(4S)], -D/iD [GlcA/IdoA(2S)-GalNAc(6S)] and –E/iE units [GlcA/IdoA-GalNAc(4S,6S)]) promote neurite outgrowth and could be valuable materials for nerve regeneration. Also displaying antiviral and anti-metastatic properties, a rare CS with fucosylated branches isolated from sea cucumbers is an anticoagulant and anti-inflammatory agent. In this same line, marine heparin extracted from shrimp and sea squirt has proven anti-inflammatory properties, with the added advantage of decreased risk of bleeding because of its low anticoagulant activity.

1. Introduction

Glycosaminoglycans (GAGs) are linear polysaccharides ubiquitous in the extracellular matrix (ECM) and the cell surface of most animal tissues, either free or bound to proteins. Once thought mere filling material, GAGs are currently recognized to be involved in a number of functions fundamental for cellular communication, differentiation and growth. Therefore, it is reasonable to conceive these molecules as potential therapeutic agents, some already realized as is the case with heparin, which has been the anticoagulant of choice for decades. Probably the most extensively investigated GAG, new roles of heparin are still presently emerging, which serves to illustrate the potential that GAGs hold.

From the chemical point of view, GAGs are polymers consisting of repeating *O*-linked disaccharide units. These units are formed by an amino sugar and a uronic acid, except keratan sulfate (KS) which has β-D-galactose (Gal) instead of the uronic acid, linked to *N*-acetyl α-D-glucosamine (GlcNAc) by alternating β1,4 and β1,3 glycosidic bonds. The amino sugar is in most cases acetylated, except in heparin, and the uronic acid can be either glucuronic acid (GlcA) or its isomer iduronic acid (IdoA) (Fig. 1). GAG chains are in most cases sulfated, except hyaluronan (HA). Sulfation in KS occurs exclusively at position 6 of the galactose and acetylglucosamine rings, whereas other sulphated GAGs display greater diversity in the position of sulfate groups. This diversity is further increased by the epimerization of the uronic acid, leading to a number of different disaccharides, in part responsible for the considerable complexity in GAG chains. Both chondroitin sulfate (CS) and dermatan sulfate (DS) contain the same amino sugar (*N*-acetyl α-D-galactosamine, GalNAc), but glucuronic acid in CS undergoes epimerization to iduronic acid in DS. Sulfation at different positions and in

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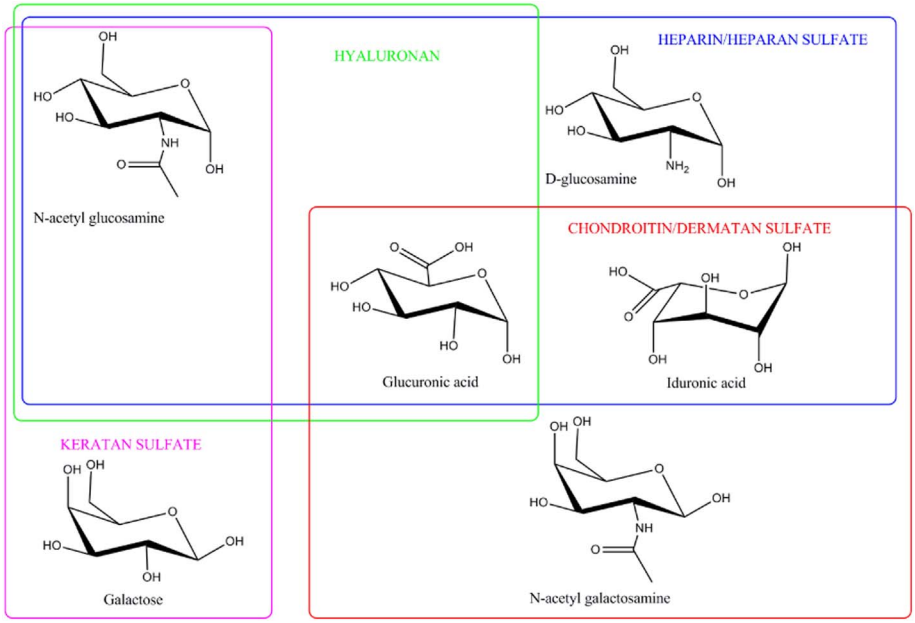

Fig. 1. Monosaccharides in GAGs.

different grades produces similar disaccharide type units in both CS and DS (Fig. 2).

Both heparin (HEP)/heparan sulfate (HS) and CS share the first biosynthetic step, but with the subsequent addition of *N*-acetyl glucosamine (GlcNAc) in the first case, instead of GalNAc. This initial disaccharide is later modified by sulfation of the aminosugar, leading to sulfoglucosamine, and epimerization of the iduronic acid. Further sulfation occurs in both the uronic acid and sulfoglucosamine, producing the most negatively charged polymer known in nature (Fig. 3). The degree and type of enzymatic modifications after polymerization lead to structural differences between HS and HEP, the latter being more extensively modified (Sugahara and Kitagawa, 2002). These differences between both polymers are mainly in terms of degree of sulfation and epimerization of the uronic ring. In HEP, the most abundant disaccharide is formed by iduronic acid and N-sulfo-α-D-glucosamine sulfated in positions 2 and 6 respectively, while in HS disaccharide abundance varies in different tissues (Shi and Zaia, 2009). Furthermore, HS chains are segregated into unsulfated and highly sulphated heparin-like domains, producing a fine structure which influences HS interactions with protein ligands (Li and Kusche-Gullberg, 2016; Rabenstein, 2002). In animal tissues, HS is expressed and excreted by most mammalian cells and is widely distributed as proteoglycans in the ECM and at the cell surface. On the other hand, HEP is stored as a glycosylation product of serglycin protein core, non-covalently bound to basic proteases, in cytoplasmatic secretory granules (Rabenstein, 2002).

GAGs can be followed throughout animal evolution, starting from primitive sponges, containing no GAGs, to humans. Along the way, some structures are widely distributed, while others are absent in lower organisms or exist with limited complexity (Yamada et al., 2011). This diversity also reflects in the different evolutionary pathways of marine and terrestrial animals, which have led to rare and sometimes unique GAGs in marine organisms. Not surprisingly, considerable effort has been placed in investigating their therapeutic properties and potential uses.

The therapeutic possibilities of GAGs are based on their effects on coagulation, thrombosis, inflammation, cancer, viral infection and tissue development. In some cases as a safer alternative to established applications, such as the anticoagulant heparin, but also with potential in tissue regeneration and in the development of antiviral and anti-tumour drugs.

The bioactivity and therapeutic properties of GAGs depend on their ability to bind to proteins, which is to a great extent influenced by sulfation (Gulati and Poluri, 2016; Hileman et al., 1998; Soares da Costa et al., 2017). Consequently, the main interest of marine GAGs resides on peculiarities related in most cases to distinct sulfation with different interrelated aspects. First, the relative abundance of sulfated units varies in marine and terrestrial GAGs (Table 1) producing changes in charge density among GAG chains. Second, rare disaccharide units, such as CS-K, are only found in marine animals (Table 1, Fig. 2) (Higashi et al., 2015a; Sugahara et al., 1996). Finally, some marine GAGs show unusual patterns (Chavante et al., 2014), specific sequences of consecutive saccharides. Particular patterns are known to be required for the binding of heparin to antithrombin (Petitou et al., 2003) and fribroblast growth factor (Capila and Linhardt, 2002), and of HS to the herpes simplex virus (Liu et al., 2002; Shukla et al., 1999). However, in most cases interactions between GAGs and proteins are not so specific and seem to be rather influenced by charge density and the presence of particular sulfated units (Li and Kusche-Gullberg, 2016; Yamada and Sugahara, 2008).

In both situations, marine sulfated GAGs add new possibilities to current terrestrial sources and they constitute the focus of the present review, incorporating recent studies using CS/DS and HEP/HS extracted exclusively from marine animals. KS, also sulfated, is found in shark cartilage (Zhang et al., 2005), zebra fish (Souza et al., 2007) and the skin of other teleost fish (Ito et al., 1984; Ralphs and Benjamin, 1992), but to the best of our knowledge, the therapeutic properties of marine KS have not been explored. Finally, hyaluronan is an interesting material in regenerative medicine (Muzzarelli et al., 2012; Prestwich, 2011) which can be isolated from the eyes of fish (Murado et al., 2012). However, marine hyaluronan is not substantially different to terrestrial sources, since lacking sulfate groups it is only defined by its molecular weight. Furthermore, hyaluronan is at present mainly produced by bacteria from the *Streptococcus zooepidemicus* species (Amado et al., 2016; Vázquez et al., 2009; Vázquez et al., 2010; Vázquez et al., 2015), making its extraction from marine organisms uneconomic. Consequently, KS and hyaluronan are not considered here.

Other polysaccharides from marine origin also show potential as therapeutic agents, including those produced by eukaryotes (alginate, fucoidans, ulvans, carrageenans, chitosan) and prokaryotes (exopolysaccharides and extracellular polymeric substances). However, these compounds lay beyond the scope of the present work and are reviewed elsewhere (Cardoso et al., 2016; Delbarre-Ladrat et al., 2014; Senni et al., 2011; Silva et al., 2012).