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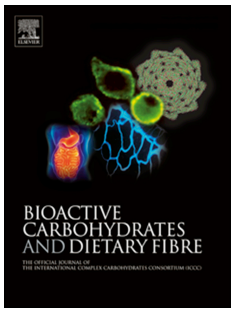

The potential of marine oligosaccharides in pharmacy

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The potential of Marine Oligosaccharides in Pharmacy

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Abstract

Complex polysaccharides are widespread within the animal kingdom. At the molecular level, however, the importance of such components have largely been considered inferior compared to nucleic acids and proteins. This in spite of the fact that carbohydrates are well known to govern important biological processes like cell-cell and cell-ECM interactions. There are probably several reasons for this, out of which the huge challenges of characterizing and synthesizing complex carbohydrates play an important role. Secondly, the fact that complex carbohydrates are not primary gene products but rather the result of post polymerization processes like e.g. glycosylation of proteins adds to the intricacy to the science of Glycobiology. As of today, heparin and heparin analogues for the treatment of DVT represent some of the very few examples of carbohydrate, or carbohydrate-inspired pharmaceutical products on the market. This review will address the complexity of carbohydrates, but also that due to this complexity carbohydrates exhibit a huge potential as carriers of information. Finally, some examples of pharmaceutically active oligosaccharides of marine origin will be presented.

Keywords

Pharmaceutical activity; epitope; glycosylation; complexity

Introduction

Biopharmaceutical drugs, aka 'biologics', are active pharmaceutical ingredients (APIs) that are directly manufactured (extracted) or semi-synthesized from biological sources. Pharmaceutical use of biologics includes the use of the bodies 'own' molecules to ameliorate disease caused by an endogenous defect in these molecules, for example insulin for diabetes mellitus or gene therapy for genetic diseases, and use of highly specific biological molecules such as antibodies to correct or control physiological processes gone awry in disease states. Over the last couple of decades, antibody therapies in particular have revolutionized treatments in areas like autoimmune diseases and cancer (Buss, Henderson, McFarlane, Shenton, & de Haan, 2012). Typically, biologics are large (>> 500 Da; oligomeric or polymeric) drugs dominated by proteins and nucleic acids. The last class of biopolymers, the carbohydrates, are not commonly found as APIs although they have found many applications as excipients and as medical devices. The reasons for this are many. Historically, carbohydrates have mainly been looked upon as structural polymers (e.g. cellulose), as entities for energy storage (e.g. starch) or as 'bulking' water-binding agents within the extracellular matrix in animals (e.g. glycosaminoglycans; GAGs). This view has prevailed even long after it became known that carbohydrates were important in cell-cell and cell-matrix interactions. Furthermore, glycosylation is a non-template process that occurs post polymerization of e.g. proteins and thereby adds another level to the complexity of biological processes, and to biological molecules, something that has often been seen as a problem rather than an opportunity. For example, unlike generic versions of small molecule drugs produced by chemical synthesis, which are structurally identical to the original drug molecule, biosimilar versions of biologics, produced under slightly different manufacturing conditions to the same genetic template may differ in glycosylation and as a result of this have different pharmaceutical effects (Revers & Furczon, 2010).

With their varying electrostatic charge, charge distribution and the presence of various non-carbohydrate substituents, carbohydrates are considered to be the most challenging biological

48 macromolecules (Mohamed & Coombe, 2017). The monosaccharides with their intricate chemistry
49 represent a challenge themselves. Compared to the basic 20 different amino acids, the simplest
50 sugars in the body can, theoretically, assemble into 4 orders of magnitude more diverse tetrameric
51 entities (Maeder, 2002). This fact will of course impose huge challenges when it comes to structural
52 analysis, not to mention synthesis, of polymeric carbohydrates, and these challenges, which
53 combine to make it difficult to elucidate the specific roles of carbohydrate structures in cellular and
54 intracellular processes, have probably contributed to the focus on bulk properties of carbohydrates
55 such as water binding.

56 The post polymerization (non-template) process of glycosylation combined with the chemical
57 complexity of carbohydrates represent a possibility to introduce a vast amount of additional
58 information in terms of functional diversity of proteins (Turnbull & Field, 2007). Figure 1 shows, on a
59 relative and logarithmic scale, to which extent additional information can be introduced through
60 glycosylation. This fact, combined with the emerging of new analytical techniques, has led to a
61 growing awareness and interest in the biological function and potential medical applications of
62 carbohydrates. The Glycome, representing the total of all carbohydrates (free or present in complex
63 molecules) in a cell/organism, and Glycomics, the systematic study of all glycan structures of a given
64 cell type or organism, are emerging scientific areas that endeavor to link the connection between
65 carbohydrates and their biological functionality.

66 Relatively speaking, carbohydrates are particularly important at higher levels than the cellular. This
67 becomes especially evident when looking at the extracellular matrix between cells, where, in
68 addition to an abundance of glycoproteins, the polymeric entities are much more often than not
69 glycosylated (GAGs on proteoglycans; (Noti & Seeberger, 2005)) . The interactions between GAGs
70 and many different proteins are essential for development and homeostasis (Kjellen & Lindahl,
71 2018). Errors in these types of interactions are linked to diseases as various as inflammation,
72 angiogenesis, neurodegeneration, cardiovascular disorders and cancer (Mohamed & Coombe, 2017).

As one of the very few carbohydrates that is used as an API, heparin stands out for the treatment of deep vein thrombosis (DVT) and has been used as a major clinical anti-coagulant since the 1930's. Heparin has the highest electrostatic charge of any biological molecule known, but the biologically active epitope of the heparin molecule is not larger than a pentamer (Figure 2; (Mohamed & Coombe, 2017; Noti & Seeberger, 2005). However, there is a large variation in chemical structure and sequence at the polymeric level so heparin's full biological function and SAR (Structure Activity Relationship) is yet to be fully understood (Noti & Seeberger, 2005). The inhomogeneous carbohydrate sequence found in heparins are very common in polysaccharides. This heterogeneity, combined with a vague specificity in carbohydrate – protein binding, point towards regulation through competitive binding / inhibition in biological processes leading to 'polypharmacy'; i.e. that identical poly- or oligosaccharides will have an effect on several biological processes and metabolic pathways (Mohamed & Coombe, 2017). As one example, it is known that heparin also possess anti-tumor properties but that has shown to be challenging to de-couple this effect from its anti-coagulation effect, and that has led to considerable research efforts into heparin mimetics (Mohamed & Coombe, 2017).

As for other biologics, systemic distribution of carbohydrates will require parenteral administration. Oligo- or polymeric carbohydrates cannot be orally administered because of stability and/or bioavailability issues. There is, however, reason to believe that unlike protein drugs, the active epitope of carbohydrates is generally rather small compared to the mother polymeric molecule, such as in the heparin case. It is intuitively clear that working with parenteral administration of oligomers rather than polymers is much more feasible because of viscosity issues and aggregation phenomena. Identification and isolation of active oligomeric epitopes hence greatly simplifies systemic distribution of bioactive carbohydrates. On the other and more challenging hand, and because oligosaccharides will originate from nature, parenteral administration requires extensive mapping of toxic and/or immunological responses. Pharmaceutical oligosaccharides have been suggested to be in the size range of 2-30 based on the degree of polymerization (Simon, 1996).

The picture of the development of carbohydrates drugs have traditionally been characterized by an extensive basic science activity at the *in vitro* level, especially in Asia (Zhang & Wang, 2015) where they have been used in traditional medicine for centuries. Human clinical investigations, on the other hand, seems to be few. One of the reasons for that may be a reluctance from large pharmaceutical companies to take in natural products combined with the lack of automated synthetic processes like there are for nucleic acids (PCR) and peptides (SPPS). There are, however, indications that this picture is about to change, especially for carbohydrates of marine origin. This review is not intended to cover all possible biological effects of marine oligosaccharides, but rather to give a few examples on actives that are already in, or are close to, clinical investigations for specific indications. For a recent and more comprehensive review on biological effects, see e.g. Vasconcelos and Pomin (Vasconcelos & Pomin, 2018).

Marine oligosaccharides

The massive marine biological diversity has for many years been looked upon as a potential source for bioactive components (Kang, Seo, & Park, 2015; Malve, 2016). Carbohydrates from marine sources exhibit similar biological functions (e.g. structural, cellular interactions, pathogen recognition) as well as similar variation in chemical composition and sequence (Vasconcelos & Pomin, 2018). However, many marine oligosaccharides, because their chemical composition are unknown to the human body, may not be encumbered with the challenge of polypharmacy linked to e.g. systemic use of GAGs like heparin from terrestrial mammals. They may therefore exhibit fewer side effects by not influencing multiple metabolic pathways because of their non-sense perception. It is also quite likely that they will not act as substrate for human trans-glycosylases and hence not become incorporated into human indigenous glycoconjugates (Varki, Cummings, & Esko, 2019).

Carrgageenan

In many incidents, the pharmaceutically active marine carbohydrates exert their pharmaceutical effect locally and do not have to be systemically administered. The antiviral properties of carrageenan is one such example. It has been shown that many bacterial, viral and parasitic pathogens use proteoglycans, in particular those containing heparan sulphate (HS), to adhere to eukaryotic cells during mucosal infections (Salvador et al., 2013). Examples of viruses that have adapted to this strategy are the common cold and influenza viruses, herpes simplex viruses (HSV), HIV, HPV and RSV (Tiwari, Maus, Sigar, Ramsey, & Shukla, 2012). Carrageenan, a sulfated polysaccharide extracted from red algae resembling HS, has shown to be an extremely potent infection inhibitor for HPV, HIV and HSV (Gonzalez, Alarcon, & Carrasco, 1987; Kilmarx et al., 2008). In clinical trials iota-carrageenan has shown to reduce the duration of disease, increased viral clearance and reduced relapses by nasal application of carrageenan spray in subjects suffering from virus-confirmed common cold (Grassauer et al., 2008; Koenighofer et al., 2014). A nasal spray containing iota-carrageenan in combination with xylometazoline HCl has been developed for the symptomatic relief of nasal congestion caused by rhinitis and sinusitis that (Graf et al., 2018).

Alginates

The linear, binary polysaccharide alginate have for decades been used as a polymer in medical devices such as wound dressings and as dental impression materials (Draget & Taylor, 2011). Alginates have also been used for symptom relief of gastric reflux (‘heartburn’) for many years under the brand name of Gaviscon. In particular application the gelling capacity, cross-linked by ions or as an alginic acid gel, is utilized by alginate forming a physical gelled barrier (‘raft’) between the gastric contents and the esophagus (Dettmar et al., 2018).

The fact that the alginate monomers, ß-d-mannuronic acid (M) and α-l-guluronic acid (G), do not occur randomly but rather in a block-wise fashion opens up for isolation of oligomers with a much higher content of either G or M than found in nature (typically > 90%). This is achieved through controlled acid hydrolysis and selective precipitation (Haug, Larsen, & Smidsrød, 1966, 1967). Such oligomers have in recent years been shown to possess pharmaceutical properties.

One pharmaceutical application of oligoguluronate is to increase the mobility of pathological mucus (Nordgard & Draget, 2011). The pathological condition *cystic fibrosis* (CF), an illness caused by a mutation in the CFTR gene, leads to a thick and static mucus in the lungs that is ideal for bacterial colonization. Mucus stasis is at least in part due to a malfunctioning mucociliary clearance. *In vitro* experiments indicated that the presence of oligoguluronates decreased the mechanical properties of CF mucus (Figure 3). A dry powder formulation of this oligomer is currently under investigation in a Phase 2b clinical trial (clinicaltrials.gov). Oligoguluronates also affect non-pathological mucus and lead to a transient opening of the mucin network pores (Nordgard, Nonstad, Olderoy, Espevik, & Draget, 2014). Mucosal delivery of pharmaceuticals, especially large entities such as biologics and nano formulations, may exhibit improved bioavailability when co-formulated with oligoguluronates (Nordgard & Draget, 2018).

It has also been shown that oligoguluronates are able to potentiate the effect of conventional antibiotics by more than two orders of magnitude on some multi-drug resistant pathogens. This effect can at least partly be explained by a disruption of bacterial biofilm formation (Rye, 2018). An *in vivo* murine breast cancer model revealed that both oligoguluronates as well as oligomannuronates exhibit anti-tumor effects (Hosseinia et al., 2017; Hosseinia et al., 2018).

Fucoidan and Laminaran

These are two poly-/oligomers isolated from brown algae and they both occur in significant amounts. Fucoidans are a family of polysaccharides rich in sulphated fucose and with considerable variation between algae species (Ale & Meyer, 2013). Laminaran (aka Laminarin) is a low molecular weight (typically 5 kDa) β-glucan storage polysaccharide (Kadam, Tiwari, & O'Donnell, 2015). Both components have shown a number of anti-cancer effects *in vitro*, such as apoptosis enhancement and inhibition of angiogenesis potential (Sanjeewa, Lee, Kim, & Jeon, 2017). A recent human clinical study investigated the efficacy of LMW fucoidan as supplemental therapy in metastatic colorectal cancer (Tsai, Tai, Huang, Chang, & Wang, 2017). This study was carried out as a double blind, randomized trial where the primary endpoint was the disease control rate (DCR). A significant improvement of DCR was observed where LMW fucoidan was administered as supplemental therapy in combination with chemotarget agents.

Conclusions

The small number of examples presented in this paper indicate that complex marine oligosaccharides indeed provide chemical compositions and sequences that can offer new possibilities in pharmacology. As carbohydrates are not commonly considered as active pharmaceutical agents there is no doubt that translational medicine becomes a central element in their development. Challenges linked to their chemical complexity, epitope identification and general ADMET considerations bespeak the need for competence in all areas from carbohydrate chemistry to clinical specialists. Their main advantage is that they represent non-sense structures that are unfamiliar to the human metabolism and as such may be able to act more specific compared to mammalian complex carbohydrates.

194

195 **Declaration of interest**

196 The authors declare no conflict of interest.

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Figure legends

Figure 1 Glycome enhancement of the molecular and functional diversity of the proteome. Reprinted by permission from Nature Chemical Biology, Nature; Emerging glycomics technologies, Jeremy E Turnbull & Robert A Field, COPYRIGHT 2007

Figure 2 Illustration of heparin and heparan sulphate structure (a) and their major/minor disaccharide repeating units (b). Unaltered from: Mohamed, S., & Coombe, D. R. (2017). Heparin Mimetics: Their Therapeutic Potential. *Pharmaceuticals, 10*(4). doi:10.3390/ph10040078 under the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/)

Figure 3 Dynamic storage modulus as function of frequency of a *cystic fibrosis* sputum sample alone and in the presence of 5 mg/ml oligoguluronates.

Figure 1

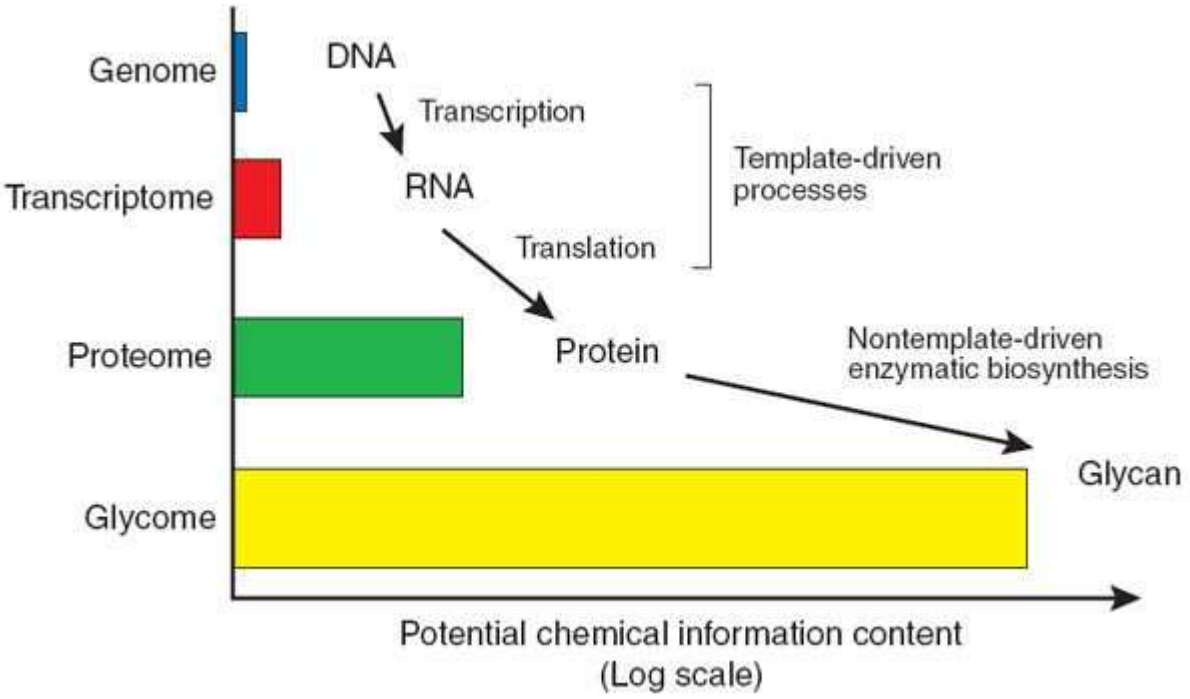
Genome
DNA
Transcription
Template-driven
processes
Transcriptome
RNA
Translation
Proteome
Protein
Nontemplate-driven
enzymatic biosynthesis
Glycan
Glycome
Potential chemical information content
(Log scale)

Figure 2

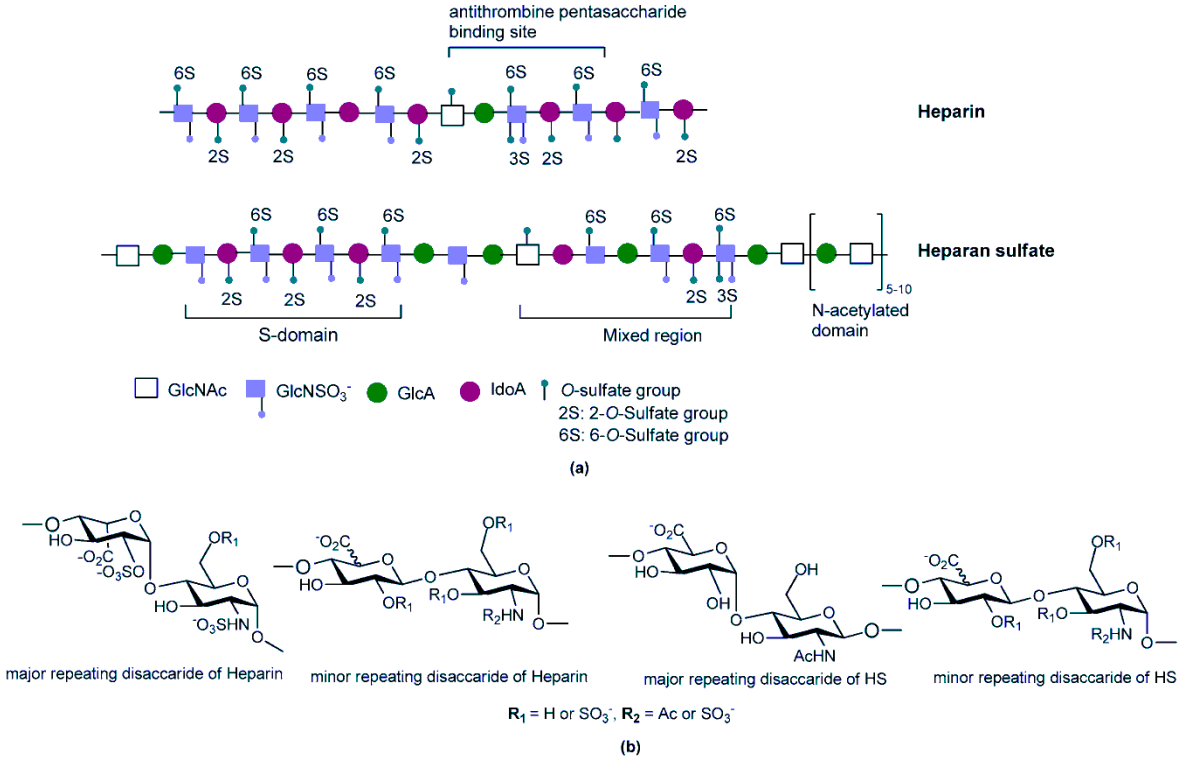
antithrombine pentasaccharide binding site
Heparin
Heparan sulfate
S-domain
Mixed region
N-acetylated domain
GlcNAc
GlcNSO3-
GlcA
IdoA
O-sulfate group
2S: 2-O-Sulfate group
6S: 6-O-Sulfate group
(a)
major repeating disaccharide of Heparin
minor repeating disaccharide of Heparin
major repeating disaccharide of HS
minor repeating disaccharide of HS
R1 = H or SO3-, R2 = Ac or SO3-
(b)

Figure 3