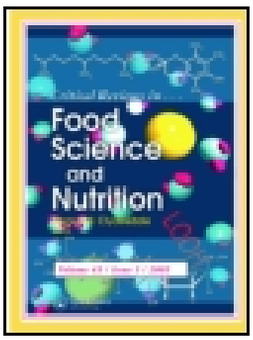

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Elaboration in type, primary structure, and bioactivity of polysaccharides derived from mollusks

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Abstract:

Over the past decades, numerous *Mollusca* species have received more attention in development and utilization as valuable bio-resources. Many efforts have been focused on investigating

mollusk polysaccharides because of their rich content, ease of extraction, diversified sorts, specific structure, various biofunctions and potent activities. To date, many mollusks, especially species of gastropods, bivalves, or cephalopods, have been reported containing polysaccharide compounds in tissues with abundant amount, and most of polysaccharides are obtainable through combining techniques of extraction, separation and purification. The polysaccharides isolated from mollusks appeared with various structural and physicochemical characteristics, ranged from neutral polysaccharides and sulfated polysaccharides, to GAGs series (including Hep/HS, CS/DS, HA and similarities), even to heterogeneous glycan with high molecular weight. This review article provides comprehensive knowledge of recent researches on type classification, tissue origins and possible biofunctions of various polysaccharides from mollusks. The highlights were placed in structure variation including molecular weight, sulfation pattern, linkages and monomer compositions for repeating unit, and primary molecular construction of the mollusks polysaccharides. In addition, this article covers general information on exhibition of mollusks polysaccharide extracts or preparations in the various bioactivities, such as anticoagulant, antiatherogenic, antioxidant, immunomodulatory, antivirus and antitumor activities, which would reveal their possible potentials in medical application. Furthermore, the article presents a brief overview on several challenges and future scope in this field.

Keywords: Mollusks, Polysaccharide, Glycosaminoglycan, Structure diversity, Bioactivities

Abbreviations

Glycosaminoglycan (GAG), heparin (Hep), heparan sulfate (HS), chondroitin (Chn), chondroitin sulfate (CS), dermatan sulfate (DS), keratan sulfate (KS), hyaluronan (HA), iduronic acid (IdoA), glucuronic acid (GlcA), galacturonic acid (GalA), glucosamine (GlcN), galactosamine (GalN), N-acetylglucosamine (GlcNAc), N-acetylgalactosamine (GalNAc), glucose (Glc), fucose (Fuc), arabinose (Ara), mannose (Man), galactose (Gal), rhamnose (Rha), xylose (Xyl), hexosamine (HexN), hexosuronic acid (HexUA), E-type of chondroitin sulfate (CS-E), molecular weight (Mw), molar percent (mol.%), weight percent (wt.%), nuclear magnetic resonance (NMR), Fourier transform infrared spectroscopy (FTIR), high performance liquid chromatography (HPLC), mass spectrum (MS), gas chromatograph (GS), international unit (IU), activated partial thromboplastin time (APTT), thrombin time (TT), prothrombin time (PT), antithrombin Ⅲ (ATIII), antithrombin Ⅱ (AT Ⅱ), half maximal inhibitory concentration (IC_{50}), nitric oxide (NO), reactive oxygen species (ROS), 1,1-diphenyl-2-pierylhydrazy (DPPH), total antioxidant capacity (T-AOC), malondialdehyde (MDA), 8-iso-prostaglandin (8-ISO-PG), glutathione peroxidase (GSH-Px), total superoxide dismutase (T-SOD), concanavalin A (Con-A), lipopolysaccharide (LPS), 2,4-dinitrofluorobenzene (DNFB), interferon (IFN), interleukin (IL), tumor necrosis factor (TNF), type-Ⅰherpes simplex virus (HSV-Ⅰ), human immunodeficiency virus (HIV), deoxyribonucleic acid (DNA), hepatitis B surface antigen (HBsAg), hepatitis B e-antigen (HBeAg), hepatitis B virus (HBV), triglyceride (TG), cluster of

differentiation (CD), alanine aminotransferase (ALT), glutamic oxalacetic transaminase (AST), cholecystokinin (CCK), calmodulin (CaM), calmodulin kinase (CaMK), cyclic adenosine monophosphate (cAMP), protein kinase A (PKA), mitogen activated protein kinase (MAPK).

1. Introduction

The phylum *Mollusca* is one of the largest, most miscellaneous and important groups in the animal kingdom, including snails, slugs and other gastropods; clams, mussels, oysters and other bivalves; squids, octopus and other cephalopods; and other lesser-known but similarly distinctive subgroups. Over 85 000 described species have been found in this phylum and the number gradually increased with year because of the new specie discovery. Mollusks are widespread in the world with huge biomass. The majority of species still live in the oceans, from the seashores to the abyssal zone, but some form a significant part of the freshwater fauna and the terrestrial ecosystems. Many mollusks species were assigned to commercial animals and have been artificially bred or fished for food consumption on account of their high nutritional value (Ponis et al., 2003) and health benefits (Celik et al., 2014). As food materials, animals in this phylum are rich and easily catchable, and thus manufactured to a variety of industrial products, and can also be developed to high valuable products, such as healthy foods, functional foods and feed additives. Except consuming as food, several species possess high medical value and have been used as folk therapeutic agents in many regions for a long period (Chakraborty et al., 2009). In traditional clinic, medical mollusks used to treat digestive diseases, neoplasm, and phlogosis or prevent senility as tonifying herbs, such as abalone and oyster, which provide officinal parts with shells (Latire et al., 2014), soft-tissues, holdfasts, mucilaginous, and even whole body (Ademolu et al., 2015). This medical convention was based on the fact that mollusks contain a lot of active ingredients including lipids, proteins, carbohydrates, mineral elements, nucleosides, srerols and so on (Ademolu et al., 2015; Anacleto et al., 2014; Grienke et al., 2014; Raghukumar, 2011). Nowadays, interest in investigation and exploitation of mollusks as natural resources has

been aroused and one major object is to extract their active component (Cesaretti et al., 2004; Karamanos et al., 1988; Lavall et al., 2007; Luppi et al., 2005a). Among all functional ingredients of mollusks, carbohydrates, especially the polysaccharide, is likely to be the most attractive compound and deserved to be persistently studied since its type and structure are diversified so much (Amornrut et al., 1999; Chavante et al., 2000; Gomes et al., 2010; Jeronimo et al., 1989; Volpi et al., 1998; Yamada et al., 2011).

Mollusk polysaccharides are obviously different with that derived from plants or higher animals in chemical composition and structures (Nader et al., 1996) on account of existing GAGs and some special polysaccharides that mostly decorated with sulfate groups (Ballance et al., 2004). GAGs and polysaccharide sulfates were synthesized as primary metabolites by mollusks and are associated with molecular recognition in cells surface and interstitial space, thereby participate in many biological processes, such as growth, tissue construction, movement, defense (Morishita et al., 2009), energy storage, environment awareness and self-regulation (Gama et al., 2006; Miller et al., 2010; Ogundipe, 2015; Pavão, 2014; Peplow, 2005). Accordingly, mollusk polysaccharides varied in types and structures to match their biological functions. These variations depend on comprehensive reasons including species, tissue origin, and developmental stage of mollusks, environment and season of harvest, and even extracting and isolating method. Undoubtedly, species and tissue origin play the most important role in decision of type and structural characterization of polysaccharides (Arumugam et al., 2009a; Arumugam et al., 2009b). It is sure that some species would generate specific type of GAG and sulfated polysaccharide (Fallis et al., 2010; Sun et al., 2010). A well-known evidence is that some mussels and clams mainly

generate glucosan analogues (Dai et al., 2009; Eckmair et al., 2016; Li et al., 2014; Wang et al., 2011b; Zhang et al., 2008) while some octopuses and snails are abundant with GAGs and proteoglycans in accumulation (Habuchi et al., 1977; Karamanos et al., 1990; Shetty et al., 2009; Wang et al., 2015b). Moreover, different species generate distinct amount of polysaccharide in their soft body despite they hold same type of polysaccharide (Arumugam et al., 2009a; Arumugam et al., 2009b; Pejler et al., 1987). Hence, after aware of this point, it is essential to summarize the rules in correlation between polysaccharide types and mollusks species for the optimal utilization of these bio-resources. However, owing to the immense species diversity of *Mollusca* and the late departure in pertinent investigation in carbohydrate biochemistry, there are relatively few mollusks have been investigated on their polysaccharides with respect to the purification and identification comparing to the total number of known species, let along summarizing systemically structural characterizations of polysaccharide in *Mollusca* (Cui et al., 2012; Karanova, 2006). Here, it must emphasize the heterogeneity of polysaccharides in this phylum because the structural diversity may even is greater than that of vertebrate, so we will discuss the analysis of polysaccharides in *Mollusca* phyla focusing on structure as well as physiological functions.

Additionally, the production and application of original polysaccharides as therapeutic agents have become increasingly important field of research (Ogundipe, 2015; Pavão, 2014; Peplow, 2005). Both GAGs and special polysaccharides from mollusks may have strong physiological activity to human. They have been evaluated in a wide range of bioactive assays, including immunomodulating, anti-inflammatory, antioxidant, and anticoagulant, antitumor and so on. Unfortunately, challenges caused by the structural diversity and heterogeneous

complicacy of mollusk polysaccharides have hinder the further development in the field of medical application (Nader et al., 1999; Volpi, 2005; Yamada et al., 2011). Giving one outline on connecting bioactivities with type and structure of mollusk polysaccharides would help to correct orientation in developing corresponding products. This article would cover general information on various bioactivities of mollusk polysaccharide extracts or preparations reported recently. Furthermore, some findings on structure-activity relationships and mechanisms of action are partly involved in this article.

2. *Mollusca* species involved in polysaccharide studies

Approximate 73 identified species of *Mollusca*, belonging to three classes, 17 orders and 29 families, are retrieved form more than 140 references, which have been studied on their polysaccharide compounds involving identification, extraction and purification, structural analysis and determination, or activity evaluation. They are listed in Table 1 and categorized into four orders of taxonomy, *i.e.*, class, order, family, and species. However, a few species listed in the table cannot be accurately identified because they were merely mentioned with trivial name in references, such as some squids (Fongmoon et al., 2007; Shetty et al., 2009), octopus (Zhang et al., 1997), mussels (Du et al., 2014) and oysters (Wang et al., 2007; Wang et al., 2006). The proportion of each category of mollusks occupied the total species were subjected to statistics according to their occurring frequency in all correlating literatures. All *Molluca* species involved in polysaccharide studies are attributed to three classes, *ie., Bivalvia, Gastropoda* and *Cephalopoda*. They occupied the prominent position in *Mollusca* phylum on account of their species diversity, resource

estimation, and economic benefit (Demaintenon, 2008; Valdovinos et al., 2010). The general information on reproduction, habitat, and biomass of all involved mollusks could also be learned from the popular scientific websites, such as Shellfish-Biodiversity Heritage Library and WMSDB-Worldwide Mollusc Species Data Base.

Bivalva is the most focused class in polysaccharide investigations and its species take approx. 55% proportion in all involved mollusks. Bivalve species are characterized with two shell valves wrapped body (Marie et al., 2008), such as mussels, oysters, scallops and clams. In *Bivalva* class, species from orders of *Veneroida, Ostreoida* and *Mytiloida* were more sought on topic of polysaccharide investigation comparing with other orders (*Adapedonta*, *Unionoida* and *Arcoida*). *Argopecten irradians*, *Ruditapes philippinarum*, *Meretrix meretrix*, and *Mactra veneriformis* (seen in Fig. 3) are the representative species in this class, which broadly distributed in intertidal zone of shallow seas of Asia, including Taiwan islands, East of China, Korea and Japan. They are popularly consumed as sea food and particularly known for their health benefits in nutrition (Zhuang, 2006; Zhuang et al., 2004).

Species in *Gastropoda* are well known for their largest amount in species (over 60 000 numbers) and most diversification in morphology and habitation, including spirally coiled snails, flat-shelled limpets, shell-less sea slugs and terrestrial snails and slugs. However, only about 24% of statistical mollusks are recognized as species of *Gastropoda* class that are interrelated in topic of polysaccharide. The relative lower proportion of *Gastropoda* is likely resulted from the low output as bio-resources because most species cannot be largely captured for commercial purpose via fishing or breeding. Species from *Gastropoda* involved in polysaccharide themes are further sum up at family level in statistics in order to avoid the taxonomic controversy in order

level. According to the statistics, several species out from 11 *Gastropoda* families have been inspected their polysaccharide compounds. However, most of them are found in three families, *Haliotidae, Achatinidae* and *Ampullaridae*, and appear with 29%, 20% and 14% occurrence in *Gastropoda* class, respectively. Species belonging to other families just have 31% occurrence in total. The most famous species from *Gastropoda* is *Haliotis discus* (seen in Fig. 2), which can be used as high price seafood and traditional Chinese medicine with huge developing potential as drugs or healthy products (Zhao et al., 2016a; Zhu et al., 2011; Zhu et al., 2010b).

Cephalopods are exclusively marine animals and are thought to be unable to live in freshwater or terrestrial. They are characterized by bilateral body symmetry, a prominent head, and a set of arms or tentacles (muscular hydrostats) modified from the primitive molluscan foot, and sometimes were called inkfish because of their universal ability to squirt ink. Mollusks in *Cephalopoda*, such as squid, cuttlefish and octopus, are the most neurologically advanced animals among all invertebrates (Liu et al., 2008a; Shetty et al., 2009; Takaya et al., 1994; Vynios et al., 1990). Additionally, either the giant squid or the colossal squid is the largest known invertebrate species. Squids and octopus are particularly known for their high nutritional value and health benefits and are usually breed or collected for food consumption. Cephalopods are less to be involved in the polysaccharide researches and merely 18% proportion of species were found in total, in which several from order of *Teuthida* were more focused by researchers (92%) since they were endowed with Chn and special proteoglycans.

3. Types and structural diversity of polysaccharides from mollusks

3. 1. Heparins and heparan sulfates

It was conspicuous that both Hep and HS belong to important members of GAG, which are major components of the extracellular matrix molecules in animal tissues and play important roles in various physiological events (Gama et al., 2006; Hoppensteadt et al., 2005; Jung, 1998; Kleene et al., 2004; Sasisekharan et al., 2002; Wang et al., 2011c). Hep and HS are distinct with other GAGs, such as Chn, DS, KS and HA, mostly lying in the disaccharide construction of chain backbone. Hep disaccharide units are made of IdoA and GlcN residues that linked with α(1→4) linkage and varied in different sulfating patterns. The most frequent disaccharide in Hep molecules is a 2-O-sulfated IdoA (IdoA2S) jointing to a 6-O-sulfated, N-sulfated glucosamine (GlcNS6S) and reveal as [4)-IdoA2S-α(1→4)-GlcNS6S-α(1→]$_n$ (Fig. 1). Most native Heps ranged their Mw from 3 to 35 kDa, but the commercial Hep prepared form beef lung or porcine intestinal mucosa are in the range of 12 and 15 kDa. HS is very closely related to Hep in structure (Fig. 1). Its frequent disaccharide unit is demonstrated as a GlcNAc linked by a GlcA via β(1→4) bond. This typical disaccharide unit of HS generally takes up around 50% parts of chain backbone. In fact, many natural HS preparations are hybrid polysaccharides since they have both ‘Hep-like’ and ‘HS-like’ moieties that are even coexisted in same molecular chain sometimes. Problems arise in defining hybrid GAGs that contain both ‘Hep-like’ and ‘HS-like’ sequence. It should qualify a hybrid GAG as Hep if its content of N-sulfate groups largely exceeds that of N-acetyl groups and the concentration of O-sulfate groups exceeds those of N-sulfate, whereas HS should be identified.

The Hep and HS type of polysaccharides are widely distributed in soft tissues of mollusks and can be acquired by series of preparing processes including extraction, precipitation, fraction, and successive purification with electrophoresis or chromatographs (Arumugam et al., 2009a; Arumugam et al., 2009b). Too many studies suggest that Hep/HS preparations purified from mollusks could be an alternative source of heparinoids due to their structural homogeneity to mammalian Hep (Arumugam et al., 2009a; Arumugam et al., 2009b; Liu et al., 2013; Periyasamy et al., 2013; Saravanan et al., 2010b; Somasundaram et al., 2007; Vidhyanandhini et al., 2014; Vijayabaskar et al., 2008; Vijayabaskar et al., 2009; Vijayabaskar et al., 2012).

Series of Hep fractions with Mw ranged from 6 to 31 kDa were obtained from clam *Katelysia opima*, *Anomalocardia brasiliana*, *Donnax striatus*, *Tivela mactroide,* and bay scallop *Argopecten irradians* and they were similar to the standard commercial Hep in chemical structure by identification (Dietrich et al., 1990; Vijayabaskar et al., 2008; Wang et al., 1994; Wang et al., 1995; Wang et al., 1996). These identifications resulted prevalently from the detection of typical Hep disaccharide that consisted of HexN (GlcN or GlcNAc) and HexUA (IdoA or GlcA), and substituted with sulfate groups, and close similarity in saccharide compositions to mammalian Hep (Wang et al., 1994; Wang et al., 1995; Wang et al., 1996). Results in structural consistency of mollusk Hep to mammalian Hep in disaccharide construction had been resorted to other species by researchers more than once. Thus a conclusion has been drawn that natural Hep GAGs isolated from mollusks were virtually indistinguishable from commercial Hep (Dietrich et al., 1990; Souza et al., 1985; Vijayabaskar et al., 2008). It has been proposed that Hep as biological

macromolecule always kept its main structural features from various origins (Ferreira et al., 1993) and through evolution (Dietrich et al., 1990). One appropriate example is that Hep isolated from marine clam *Tapes phylippinarum* (Table 2), which owns 80.6 mol.% of ΔIdoA2S-GlcN2S6S, 4 mol.% of ΔGlcA-GlcN2S6S, 6 mol.% of ΔIdoA2S-GlcN2S, 1.8 mol.% of ΔIdoA-GlcNAc6S, 2.3 mol.% of ΔGlcA-GlcN2S3S6S and 0.2 mol.% of ΔIdoA-GlcN2S6S in disaccharide compositions, and is closely similar to that of bovine mucosal Hep (Cesaretti et al., 2004). Another Hep sample from *Anodonta anodonta* (Table 2) showed sulfating degree similar to that of bovine mucosal Hep because of the substantial presence of same molar proportion of the trisulfated disaccharide of ΔIdoA2S-GlcN2S6S (77.5%), a slight modification of the other oligosaccharides of ΔGlcA-GlcN2S6S (4.7%), ΔIdoA2S-GlcN2S (8.6%) and ΔIdoA-GlcNAc6S (1.3%), and a significant increase of the tri-sulfated disaccharide emerging the sulfate groups in amino of C-2, and hydroxyls of C-3 and C-6 of GlcN demonstrating with ΔGlcA-GlcN2S3S6S (2.9%) as the core part binding to AT-Ⅲ(Volpi et al., 2005). However, other reports gave different proofs that Hep GAGs isolated from mollusks were likely to vary and formed their own unique structural characterizations in disaccharide compositions, sulfating pattern, linkages and Mw, according to source of species. Series of Hep GAGs were purified from two calms of *Anomalocardia brasiliana* and *Tivela mactroides* and identified to be high sulfated GAGs (Table 2). All of them contain higher amounts of 3-O-sulfated disaccharide sequences, representing as ΔGlcA-GlcN2S3S and ΔGlcA-GlcN2S3S6S, which take up to 25 ~ 30% in molar composition of total disaccharides. A novel disaccharide sequence, displaying as ΔIdA-GlcN2S3S6S that linked to GlcN2S residue in chain, had also been found with a low amount (3 ~ 4%) (Pejler et al., 1987). Hep presented with approx. 1.9 mg/g of content in tissue of *Callista chione* is a

low-sulfated polysaccharides and possess about 10.9 kDa of average Mw (Table 2). Structural investigation revealed the presence of low amounts of the trisulfated disaccharide, existing as ΔIdoA2S-GlcN2S6S (40.8%), and a significant increase of the disaccharides containing non-sulfated HexUA (IdoA or GlcA), showing with ΔIdoA-GlcNAc6S (2.1%), ΔIdoA-GlcN2S6S (11.8%) and ΔGlcA-GlcN2S3S6S (1.4%) (Luppi et al., 2005a; Luppi et al., 2005b).

Researchers also estimated that structure of HS compounds from mollusks *of Anomalocardia brasiliana*, *Tagelus gibbus* and *Pomacea* sp. at early stage (Souza et al., 1985). Likewise, these mollusk HS GAGs were proved very similar to mammalian HS in structure. Thereafter, structure of HS isolated from *Pomacea sp* were reported in detail and compared with the most commercial HS described so far in disaccharide composition. HS from *Pomacea sp.* were degraded by heparinases I and II to four distinct unsaturated disaccharides and performed with ΔGlcA-GlcNS6S, ΔGlcA-GlcNS, ΔGlcA-GalNAc6S and ΔGlcA-GalNAc (Table 2). However, HS isolated from eggs of *Pomacea sp.* at different stages demonstrate higher value of Mw than that extracted from adult snail. The primary structure in proportion of the four disaccharide units in HS of eggs varied according to the stage of development (Jeronimo et al., 1989). Ferreira et al. once again paid attention to the HS extract from *Anomantidae sp.* and compared it with mammalian derivatives (Table 2). The HS from *Anomantidae sp.* is composed of different oligosaccharide blocks of ΔGlcA-GlcNAc, ΔGlcA-GlcN2S and ΔGlcA-GlcN2S6S. Its non-reducing end was a monosaccharide of GlcN2S6S while the reducing ends was a disaccharide of ΔGlcA-GlcNAc6S (Ferreira et al., 1993). An unusual type of HS GAG found in *Anodonta*

californiensis is extremely rich in non-sulfated ΔIdoA-GlcNAc residues and very similar to the structure of HS isolated from lobsters (Hovingh et al., 1993). Recently, one unique HS-like polymer has been isolated from the bivalve *Nodipecten nodosus* (Table 2). Its disaccharide units in chain are of GlcA (non-sulfated), GlcA2S or GlcA3S that randomly linked by GlcN2S, GlcNAc6S or GlcN2S6S via β(1→4)-linkage. This HS is plentiful in soft body of *Nodipecten nodosus* and has approx. 4.6 mg/g of content (Gomes et al., 2010). The disaccharide composition of one HS sample from *Amussium pleuronectus* was analyzed like porcine intestinal mucosal HS and manifested in typical sulfated patterns, seeing that it processed equivalent amount of HexUA and HexN (Saravanan et al., 2010a).

A new GAG preparation isolated from the giant African snail *Achatina fulica* should be classified into HS family and named as acharan sulfate (Jeong et al., 2001). Acharan sulfate has over 29 kD of Mw and occupied 3 ~ 5% of total dry weight of soft body in the snail (Kim et al., 1996). The uniform repeating disaccharide found in acharan sulfate structure is a IdoA residue linking to a GlcNAc residue through α(1→4)-glucosidic bond (Table 2). Thereby, many such disaccharide units (ΔIdoA-GlcNAc) connect each other by α(1→4)-bonds from C-1 of GlcNAc to C-4 of IdoA and form linear like chains of acharan sulfate. The molecular chains of acharan sulfate are terminated by 4-linked α-D-GlcNAc residue at the reducing end and by 4,5-unsaturated HexUA2S at the non-reducing end (Chi et al., 2006). Sulfate groups are invariably substituted to the hydroxyl of C-2 position of IdoA residues. According to the sulfation, acharan sulfate usually includes two series of sequences in chain, one is ΔIdoA2S-GlcNAc as major repeating unit and the other is ΔIdoA-GlcNAc without any presence of sulfating group as minor parts. Structurally, acharan sulfate is geared to the HS family of

GAG, but is distinctly different from all known members of these classes of GAGs (Kim et al., 1998).

Several studies more focused on the emergence and biological function of Hep/HS rather than the accurate structural analysis (Hillman, 1968, 1969). These investigations were further extended to different tissues of several specific mollusks, such as *Anodonta californiensis* (Hovingh et al., 1993) *and Mercenaria mercenaria* (Ulrich et al., 2001). Histochemical identification and histogenesis of Hep/HS in various tissue of a freshwater mussel *(Anodonta californiensis)* showed that the biomacromolecules (Hep/HS) were mainly present in exterior pericellular and basement membrane locations of mussel's gill and mantle. Gill produced about 50% of each polymer while mantle synthesized minor Hep but major HS compounds. It was speculated that HS/Hep as acidic polysaccharides play a potential biofunction in calcium transport (Hovingh et al., 1993; Wada et al., 1971) or storage for growth and development of mollusks (Zhou et al., 2013). Another survey on how the Hep distributed in tissues of the northern quahog clam (*Mercenaria mercenaria*) demonstrated that Hep extensively occurred in several morphological cell subpopulations of the labial palp, ctenidia, intestine, siphon, and pallium tissues. Concentrated dyeing Hep molecules were observed in the form of granules inside organelles in the cytoplasm of several special cells. Mast-like cells containing Hep were restricted to areas proximal to epithelial surfaces. It was the "mast-like" cells seized of high amount of Hep that possibly played a key role in inflammatory responses and could analogize to the mammalian mast cells (Ulrich et al., 2001). These studies ultimately led to one proposal that HS would take part in the cell-cell recognition phenomena, growth control of cell and mineralization of

shell, whereas Hep is likely involved in defense mechanism against bacteria and other foreign materials (Hovingh et al., 1993; Kim et al., 1998; Rupavathi et al., 1984).

3. 2. Chondroitin and dermatan sulfate

CS and DS comprise Chn family of GAG and are the most prevalent animal polysaccharides in nature world. The repeating disaccharide units are comprised of a HexUA sugar (GlcA or IdoA) and a GalNAc sugar by linkage of β(1→3) bond and companied with or without sulfation, in this way the disaccharides randomly connect each other with β(1→4) bonds to generate the liner polymeric chains of CS or DS (Fig. 1). DS only differentiate CS in disaccharide by replacement of IdoA with GlcA epimer.

The Chn GAGs could be well prepared from the byproducts of some commercial squid species that belong to *Teuthida* order of *Cephalopoda* class, including skin, cartilage, cornea, liver integument, even squid ink. However, the prepared squid Chn GAGs have variability in chain compositions, Mw and sulfating manner with respect to their tissue origins. Karamanos has isolated Chn containing proteoglycans from squid skin then identified that the polysaccharide parts release from core proteins includes non-sulfated Chn, over sulfated Chn and normal sulfated Chn (Karamanos et al., 1988). Two or four such Chn chains attached to core protein and form two populations of proteoglycans with 4.8×10^2 and 2.8×10^2 kDa of Mw, respectively (Karamanos et al., 1990). Similar Chn conjunct proteoglycans have been isolated from squid cranial cartilage. Unlike that from squid skin, proteoglycans obtained from squid cranial cartilage were attached by two to five Chn chains via an O-glycosidic bond involving GalNAc and most likely Xyl residues. These polysaccharide chains are part of over sulfated Chn thanks to

containing much sulfated disaccharide residues in molecules (Vynios et al., 1990). Three types of Chn from squid cornea were discovered by Karamanos et al. and belonged to over-sulfated Chn, normal Chn and low sulfated Chn, sharing 93%, 5% and trace proportions in crude extracts, respectively. The over sulfated Chn from squid cornea contain 52% of ΔGlcA-GalNAc4S, 28% of ΔGlcA-GalNAc4S6S, 9% of ΔGlcA-GalNAc6S and 11% of ΔGlcA-GalNAc sequences in molar composition of disaccharides (Table 3). In contrast, molecular chain of the normal sulfated Chn from squid cornea are consisted of 49% of ΔGlcA-GalNAc4S, 30% of ΔGlcA-GalNAc, 20% of ΔGlcA-GalNAc6S and 1% of ΔGlcA-GalNAc4S6S (Karamanos et al., 1991). The compositional analysis of one novel Chn compound purified from squid liver integument (Table 3) revealed a range of variably sulfated disaccharides with ΔGlcA-GalNAc6S, ΔGlcA-GalNAc4S, and ΔGlcA-GalNAc4S6S as the major structural units, significant amounts of rare GlcA3S containing disaccharides, and a less amount of nonsulfated disaccharide as ΔGlcA-GalNAc (Shetty et al., 2009).

The over sulfated Chn from squid tissue, especially from cartilage, were recognized as CS-E series because of containing the so-called E disaccharide unit ΔGlcA-GalNAc4S6S, which was used to distinguishing with CS-K series (other CS isolated from king crab cartilage and was rich in GlcA3S residue in disaccharide) (Kinoshita et al., 1997). Enzymatic digestion of CS-E to produce specified oligosaccharide sequence manifested that several disaccharides in variable sulfating pattern coexisted in same polymeric chain at early stage, including ΔGlcA-GalNAc4S, ΔGlcA-GalNAc6S and ΔGlcA-GalNAc4S6S (Karamanos et al., 1991; Kinoshita et al., 2001). CS-E from squid cartilage also contained

rare GlcA3S residues adjacent to the non-reducing end of chains. GlcA3S residues just present in two or three successive disaccharide units closing to chain end. The predominant structure on the nearest non-reducing side of CS-E (80%) was a GlcA3S residue connecting to GalNAc4S (ΔGlcA3S-GalNAc4S), whereas that in the reducing side was GalNAc4S6S (59%). Shortly after, it was further proposed occurrence of internal GlcA3S residues in chain of CS-E from squid cartilage. Internal GlcA3S residues are not clustered but rather interspersed in the CS-E chains, being preferentially located in the highly sulfated sequences. The highly sulfated sequences are made up of disulfated and trisulfated disaccharide units that respectively appeared as ΔGlcA3S-GalNAc6S and ΔGlcA3S-GalNAc4S6S (Fongmoon et al., 2007). These disaccharide units are novel and unusual, and are distinct from other CS isoforms (Table 3). In the chain of CS-E, approx. 5 mol. % of total disaccharide units were substituted with Glc at the C-6 position of the constituent GalNAc residues by β(1→6) linkages. Sugars of Glc presenting as branch of CS-E make its structure was definitely different with the commercial CS from mammals (Habuchi et al., 1977; Karamanos et al., 1991; Kinoshita et al., 2004). Higashi et al. reported that CS containing 13.9% of K-type units (ΔGlcA3S-GalNAc4S) was found in octopus (*Enteroctopus dofleini*). However, proportion of fucosylated disaccharide units in this octopus CS was very low (Higashi et al., 2015). Non-sulfated Chn from squid animals were also concerned in its structural study. Actually, non-sulfated Chn is widespread in various squid soft tissues (Junichi et al., 2009). Squid ink is usually discarded with little commercial use but generate non-sulfated Chn likewise (Matsue et al., 1997; Takaya et al., 1994). Non-sulfated Chn from squid ink appears with equal molar of GlcA, Fuc and GalNAc residues and has been demonstrated a unique structure (Table 3) with that Fuc residue of the disaccharide (ΔGlcA-Fuc)

in chain being substituted by GalNAc sugar as branch at C-3 position via α(1→3) glycosidic bond (Matsue et al., 1997). Similar non-sulfated Chn were also found in ink sac of the squid *Ommastrephes bartrami* (Table 3). Chen has elucidated that the structure of Chn from ink sac contained repeating disaccharide of ΔGlcA-β(1→4)-Fuc in chain and integrated with the GalNAc as branches that substituted at C-3 position of Fuc residue by α(1→3)-linkage (Chen et al., 2008). The two non-sulfated Chn GAGs obtained from squid ink and squid ink sac have same chain structure. However, ink of cuttlefish *Sepiella maindroni* has another non-sulfated polysaccharide that is distinct with the above two. It is not certain whether this polysaccharide belong to Chn compounds just by current consequence of structural investigation. This none sulfated polysaccharide is made up of GlcA, Man, GalNAc, and Fuc in monosaccharide molar ratio of 1:1:2:2, and possesses 11.3 kDa of average Mw. The chain backbone of polysaccharide consist of Fuc, GalNAc and Man in a molar ratio of 2:2:1, on conditions that GlcA as branches attached to Man residues via α(1→3)-linkage (Liu et al., 2008a).

In fact, the Chn GAGs are prevalent in mollusks instead of merely funded in cephalopods. There are also too many bivalves and snails been detected the presence of CS polysaccharides. Study of Cao et al. provide one proof to support this conclusion. They confirm that Chn existence in 20 *Mollusca* species, including 15 bivalves and 5 gastropods. The universal presence of Chn in mollusks was detected through resolving the specific disaccharide of ΔGlcA-(1→3)-GalN with PMP-HPLC-MS^n after the acid hydrolysis of extracts of the bivalves and snails (Cao et al., 2015). However, the study cannot give more structural information of Chn GAGs that derived from or presented in bivalves and

gastropods. Defined structure study on Chn GAGs in bivalves and snails was implicated into the species of *Anomalocardia brasiliana, Tagelus gibbus, Pomace*a sp. and *Planorbarius corneus.* Those species were reported containing Chn compounds that have similar structure with the standard CS prepared from mammals (Souza et al., 1985; Volpi et al., 2007). It was reported that Chn are detectable in whole course of embryonic development of *Pomacea sp*. (Table 3). However, Chn purified from the eggs of *Pomacea sp.* excludes the 6-O-sulfated disaccharide (ΔGlcA-GalNAc6S) and IdoA residues but owns common Chn structural features (Jeronimo et al., 1989). The large freshwater mollusk bivalve *Anodonta anodonta* also contains Chn compounds (Table 3). GAG extract from *Anodonta anodonta* approx. includes 38% of sulfated Chn and 21% of nonsulfated Chn. The purified sulfated Chn from *Anodonta anodonta* comprised approx. 28% of the 6-sulfated disaccharide (ΔGlcA-GalNAc6S), 46% of the 4-sulfated disaccharide (ΔGlcA-GalNAc4S), and about 26% of the nonsulfated disaccharide (ΔGlcA-GalNAc), with a charge density value of 0.74 (Volpi et al., 2005).

Unlike the above Chn compounds, DS in mollusks has been paid few attentions. As a case in point, one DS purified from the marine clam *Scapharca inaequivalvis* was carried out for sufficient structural characterization (Table 3). The DS was discovered comprising approx. 75% of IdoA containing disaccharides (ΔIdoA-GalNAc) and 25% of GlcA containing disaccharides (ΔGlcA-GalNAc). Disaccharides of ΔIdoA-GalNAc are mostly monosulfated in C-4 position of GalNAc and/or partly disulfated in C-2 of the IdoA and C-4 of GalNAc. In contrast, disaccharides of ΔGlcA-GalNAc are more associated with nonsulfated pattern despite that some in low percentages appear with monosulfated pattern (ΔGlcA-GalNAc4S or ΔGlcA-GalNAc6S) and preferentially locate inside the chain. Generally speaking, this DS possesses a peculiar

structure in its carbohydrate backbone, most lying in the presence of significant amount of nonsulfated disaccharides located close to the nonreducing end, the elevated percentage of disaccharide of ΔIdoA2S-GalNAc4S, and the presence of low amounts of IdoA2S containing disaccharide. *S. inaequivalvis* DS was also found to hold a mean Mw of approx. 27 kDa and a mean charge density of 1.10 (Volpi et al., 2009).

3. 3. Hyaluronic acid

Hyaluronan (or hyaluronic acid, HA), another member of GAGs, belong to a non-sulfated linear polysaccharide. Its chain is composed by repeating disaccharide units of Δ GlcA-GlcNAc and can be drawn as [4)-GlcA-β(1→3)-GlcNAc-β(1→]$_n$ (Fig. 1). HA GAGs were widespread found in various species that ranged from bacteria to vertebrates, and in all tissues or body fluids, especially the loose connective tissue of vertebrates. HA is synthesized in the cellular plasma membrane and excreted to extracellular matrix in the formation of polyanions with very high Mw, ranged from 100 to 10 000 kDa. As one of the chief components of the extracellular matrix, HA is associated with binding cell surface to other matrix components and contributes significantly to cell proliferation and migration (Fraser et al., 1997). Notably, the occurrence of HA is restricted to a few species of mollusk within the scope of invertebrates, but biological significance of HA in mollusks has not been clarified yet. Previously it was very difficult to demonstrate the presence of HA in mollusks because it could not be distinguished from non-sulfated Chn owing to their structural similarity. HA and non-sulfated Chn are identical in the sugar stereo configuration, the substitution pattern of the backbone hydroxy groups and glycosidic

linkages. The mere difference in structure between them is the configuration at the C4-position of the HexN residue, exhibiting as GlcNAc or GalNAc. However, using modern techniques including NMR, HPLC and capillary electrophoresis, HA in the mollusk is clearly identifiable.

So far, HA has been found in bivalve *Mytilus galloprovincialis*, *Anodonta cygnea*, *Chlamys farreri* and *Argopecten irradisus*. The HA produced by mollusk *Mytilus galloprovincialis* can generate typical disaccharide of hyaluronan with ΔHexUA-GlcNAc after digestion with chondroitin ABC lyase. HA from *M. galloprovincialis* was determined with 200 kDa of average Mw, which is much smaller than average Mw of mammalian HA (1000 ~ 10 000 kDa), and had a very low specific viscosity. It was also unable to interact with aggrecan from bovine cartilage in formation of high Mw of aggregate (Volpi et al., 2003). GAGs isolated from the leftover bits of the *Chlamys farreri* and *Argopecten irradisus* were identified to be HA molecules in the same way but they were observed with minute content of Gal, Glc, Man, Rha, Fuc and Xyl in monosaccharide composition (Li et al., 2004). HA also was found occurring in the haemolymph and extrapallial fluid of mollusk *Anodonta cygnea* and coexisting with HS type of GAG in order to play an important role in the process of nacreous shell biomineralization (Lopes-Lima et al., 2005).

3. 4. GAG similar polysaccharides

There are other several GAGs similar compounds occurring in mollusks instead of the normal GAGs mentioned above, i.e. Hep, HS, Chn, Ds and HA. These polysaccharides occurred in mollusks share similarity with normal GAGs in structure or physicochemical property. As for structure, they are linear or branching polysaccharides that main chains are made up of repeating

oligosaccharide units. The repeating units consist of at least one alternating amino sugar (HexN) or at least one alternating uronic sugar (HexUA). Sugars in repeat units generally were sulfated with different patterns and therefore feature them with polyelectrolyte properties.

A novel glycosaminoglycan-like sulfated polysaccharide (APP) was purified from the pleopods of *Haliotis discus hannai* Ino. Chemical composition analysis indicated that APP was constituted by GalNAc, GlcA, Fuc, Gal with a ratio of 2.14:2.37:2.94:1. APP contains approx. 15.5 wt.% of sulfate and possesses 56.2 kDa of average Mw, expressed as a branched polysaccharide. Its main chain was proposed with [→3)-GalNAc-β(1→2)-GlcA-β(1→3)-GalNAc-β(1→4)-GlcA-β(1→]$_n$ (Fig. 2). In this backbone, Fuc-α(1→ and Gal-α(1→2)-Fuc4S-α(1→ as branches were linked to C-4 and C-5 positions of the first GalNAc residue, while another branch of Fuc3S4S was linked to 4-O position of the second GalNAc residue of the repeating unit via α(1→4) bond (Li et al., 2011). Abalone GAG-like polysaccharide with the repeating unit of [→3)-GalNAc-β(1→2)-GlcA-β(1→3)-GalNAc-β(1→4)-GlcA-β(1→] as main backbone was not only verified its presence in pleopod of *Haliotis discus hannai Ino*, but also discovered in pleoped, gonad and viscera of another *Gastropoda* mollusk, *Volutharpa ampullacea perryi* (Wang et al., 2015b). However, it was noticeable that the APP wasn't the only one GAG similar polysaccharide detected in abalone mollusks. Abalone gonad sulfated polysaccharide (AGSP) had been discovered in gonads and viscera of *Haliotis* and in pleopods, gonads and viscera of *Volutharpa* as well. AGSP is of HexUA-containing polysaccharides and consist of the specified disaccharide unit characterizing as

ΔGlcA-β(1→2)-Man. The backbone of AGSP are composed of [→4)-GlcA-β(1→2)-Man-α(1→] repeating unit (Wang et al., 2015a; Wang et al., 2015b). One papain-released polysaccharide designated CFPS-2 was isolated form the fresh water clam *Corbicula fluminea.* Chemical composition analysis indicated that CFPS-2 included sugars of GlcN, Glc, Gal, Fuc and sulfate groups, with an average Mw of about 22 kDa. It was speculated that CFPS-2 should be a GAG similar polysaccharide according to its structural information (Liao et al., 2013). Zhang has purified a novel oligosaccharide chain from octopus rhodopsin that appeared with backbone structure of Gal-β(1→3)-GlcNAc-β(1→2)-Man-α(1→3)-Man-β(1→4)-GlcNAc-β(1→4)-GlcNAc. This structure is quite unique because a novel residue of Gal-β(1→4)-Fuc attaches to C-6 position of the reducing terminal GlcNAc residue by α(1→6) linkage and a terminal Man residue attaches to C-6 position of the second Man sugar in main chain by α(1→6) linkage. Although the oligosaccharide chain from octopus rhodopsin showed distinct structural characteristics when was compared with the existing GAGs, we still incline to attribute it into the type of GAG similar polysaccharide since it has the GlcNAc residues. The structural drawing of this novel branching oligosaccharide was demonstrated in Fig. 2 (Zhang et al., 1997).

3. 5. Chitin from hard tissues of mollusks

Chitin is a long-chain polysaccharide composed by the GlcNAc residues, and found in many places throughout the natural world. It is a characteristic component of the exoskeletons of arthropods such as crustaceans and insects, and also exist in the radulae and internal shells of bivalves (Teanchai et al., 2016), as well as the pen beaks of squids (Jung, 2013). The units of

GlcNAc in chitin connect through covalent β(1→4)-linkages, resulting to structure of chitin are very similar to cellulose. Chitin may be described as cellulose with one hydroxyl group on each monomer replaced with an acetyl amine group. Like cellulose, the rigid chain of chitin allows for increased hydrogen bonding between adjacent molecules, forming crystalline nanofibrils or whiskers to provide rigid mechanical property or paly supporting role for sclerous tissues after incorporating with calcium mineral salts and collagen proteins.

Chitin has been isolated from the pens of many squids including species of *Loligo sanpaulensis* (Lavall et al., 2007), *Loligo plei* (Lavall et al., 2007), *Loligo lessoniana* (Chandumpai et al., 2004), *Loligo formosana* (Chandumpai et al., 2004), *Loligo vulgaris* (Ianiro et al., 2014), *Loligo chenisis* (Cuong et al., 2016), *Illex argentines* (Cortizo et al., 2008), *Dosidicus gigas* (Jung, 2013; Jung et al., 2011; Jung et al., 2014), *Todarodes pacifica* (Youn et al., 2013) and so on. The chitin isolated from squid pens was identified to be β-chitin, which were allomorph of α-chitin isolated from crab shell. The β crystal type of chitin was caused by different intramolecular and intermolecular hydrogen bonds in comparison with α-chitin. Totally speaking, crystal of β-chitin consist of two chains paralleling to the same direction, while crystal of α-chitin are made up of two chains paralleling to the opposite direction. Thus β- and α- crystal formation brings huge variation in chemical and physiochemical properties to chitin, such as deacetylating reaction, substitution reaction (Dong et al., 2001), degradation velocity (Jung et al., 2011), dye-binding capacity (Youn et al., 2013), moisture absorption ability (Jung et al., 2014) and strength of fabrications (Kumar et al., 2013). Isolating β-chitin form squids usually underwent sequential treatments of demineralization and deproteinization (Youn et al.,

2013). The obtained β-chitin from squid pens were ranged in yield from 25.5% to 38%, had higher Mw, contained little contents of ash and metals, and combined with a negligible amount of proteins. Considering the low economic value and the character of fishery waste, the pens and endoconchs of squids are undoubtedly excellent source to extract the β-chitin materials. Chitin was also found presenting in mollusk shells, such as nacre of abalone (Weiss et al., 2002) and *Hyriopsis cumingii* (Zheng et al., 2015). Chitin is one of the most important organic substances in the interlamellar matrix and arranged in a well-organized manner to build up the nacreous layer. Chitin fibers in nacreous layer are aligned with certain mineralogical axes of crystalline calcium carbonate in a species-specific manner (Weiss et al., 2002).

3. 6. Glucan compounds

Special glucans excluding glycogen and presenting in mollusks, especially the bivalve species, have been recorded for a long time and still been focused today by many scholars (Eckmair et al., 2016). The glucans have variable structure in linkage ways of sugar residue, monosaccharide composition, chain construction, Mw, and even chain conformation, but it is still unknown how they are generated in mollusks and what their biological role is. Early study on mollusks glucans were reported by Lash et al. that involves one glucan sulfate isolated from the marine snail *Busycon caniculatum*. This glucan sulfate was found coexisting with other two GAGs (CS and Hep) in rasping organ (radula) of the snail and appearing with about 35 wt.% of sulfate content. The sulfated polysaccharide was identified to be polyglucose because its complete hydrolysis generated only D-Glc sugar and was found no trace of uronic acid or amino

sugar. However, the entire structure of this sulfated polyglucose had not been more elucidated (Lash et al., 1960).

A novel glucan fraction was isolated from the mantle of the scallop *Patinopecten yessoensis,* which was possibly a homogeneous proteoglycan complex from deduction of gel-filtration and ultracentrifugation results since it contained 83 wt.% of glucan and 13 wt.% of proteins. Its average Mw was estimated to be 40 kDa by means of molecular-sieve chromatography on Sepharose CL-4B. The structural information from methylation studies, periodate oxidation, amylolysis, NMR spectral data, elucidated that the linear backbone of polysaccharide part consisted of α(1→4)-Glc units by successively linking. Several D-Glc sugars were attached to every fourth sugar unit of the backbone through α(1→6) or by α(1→3) linkages as branches. This glucan is obviously different with the glycogen compounds according to recent generating structure (Molchanova et al., 1992).

Two water-soluble polysaccharides, PEF1 and PEF2, were purified from aqueous extract of a marine mollusk, *Ruditapes philippinarum*. The chemical structures determined by FTIR, GC, HPLC and NMR indicated that both PEF1 and PEF2 were homo-glucan/protein complexes with 26 and 8.2 wt.% of protein contents, and their average Mw were about 2000 and 5 kDa, respectively. The glucan moiety of PEF1 was mainly α(1→6)-branched α(1→4)-D-glucan (a glycogen analogue), while that of PEF2 covered both α(1→4)-glucan and β(1→6)-glucan (Zhang et al., 2008).

Another water-soluble polysaccharide HCLPS-1 was isolated from clam of *Hyriopsis cumingii* Lea by hot water extraction and purified with DEAE-cellulose and

Sephadex-G200 gel-permeation chromatographs. HCLPS-1 consisted of Glc and Xyl with the molar ratio of 35:1 and had a weight-average Mw of about 156 kDa. Based on the structural data obtained from methylation analysis, GC-MS and NMR spectroscopy, the main chain of HCLPS-1 (Fig. 3) is composed of Glc and Xyl residues that linked by β(1→4)-linkage. Xyl residues are occasionally inserted in main chain of polysaccharide, while two branches of β(1→4)-glucan with variable polymerizing degree linked to Glc residues distributed both side of Xyl residue in backbone by β(1→6) linkage, constructing the well-organized branching polysaccharide of HCLPS-1 (Dai et al., 2009).

Recently, our researching team also devote to investigate the special polysaccharide from mollusks (Wang et al., 2011a). We have successfully purified some homogeneous glucans (MVPS) from the crude extract of *Mactra veneriformi* by DEAE-cellulose column chromatography. The glucans appear with about 450 kDa of average Mw and all of them only contained D-glucose residues in monosaccharide analysis. One glucan ingredient named MVPS-2 was selected to characterize its absolute chemical structure by NMR analysis. Result showed that MVPS-2 was a liner polysaccharide and D-Glc residues were repeatedly connected by α(1→4) and α(1→2) linkages with 6:1 molar ratio appearing in its backbone (Wang et al., 2011b). This structure (Fig. 3) brought MVPS the flexible chain characteristics. Glucans of MVPS were non-crystal but thermostable, and could easily dissolve in aqueous media (Wang et al., 2016). The relevant polymer solution obviously belonged to non-Newtonian fluids even at relatively high concentration (Wang et al., 2013).

One water-soluble polysaccharide MMPX-B2 was isolated from *Meretrix meretrix* Linnaeus (Fig. 3). MMPX-B2 consisted of Glc and Gal residues at a molar ratio of 3.5:1 approx. 510 kDa of average Mw. MMPX-B2 possessed a main chain of α(1→4)-linked-Glc residues, partially substituted at the hydroxyl of C-6 position in Glc residue by a few terminal Gal residues or branched chains consisting of β(1→3)-linked Gal residues (Li et al., 2014).

Sinonovacula constricta has been widely used as a health food and medicine in China, Japan, and Korea and it contains ployglucose compounds. Luan et al. have isolated one soluble glucan from *Sinonovacula Constricta* named YC-S2 (Fig. 3). The relative Mw of YC-S2 determined by HPLC was 482 kDa. YC-S2 was characterized to be a branching glucan structure that displayed its main chain with α(1→4)- glucan and a little branches with β(1→4) glucan. The branches attached to the main chain through β(1→3) linkages (Luan et al., 2015). Another water-soluble polysaccharide fraction (SCP-1) has also been prepared from *Sinonovacula constricta* by enzyme-assisted extraction and purification of chromatography with DEAE-52 cellulose anion-exchange column and Sephadex G-100 size exclusion column. However, SCP-1 (Fig. 3) has different structure with the above glucan of YC-S2. On the basis of the analytical results of HPLC, FTIR spectroscopy, methylation analysis, and NMR spectroscopy, SCP-1 was found to have an average Mw of 15.6 kDa and a linear backbone of α(1→4) linked -D-Glc residues with one branch, α-D-Glc, attached to the main chain by a α(1→6) glycosidic bond at every five α-D-Glc units (Yuan et al., 2015).

3. 7. Galactan compounds

Galactan is another type of polysaccharide dislike GAG that found in mollusks, especially in some snail's species of gastropods. One galactan elaborated by Duarte et al. from the albumen glands of the snail, *Strophocheilus oblongus*, is composed mainly, but not entirely, of β-linked-Gal residues (Fig. 4). The galactan was proved to be a highly branched polysaccharide through fully methylation analysis, since its molecular backbone contained high proportion of terminal Gal residues (43 mol.%) and junction residues of β(1→3,6)-Gal (43 mol.%) as well as with low proportion of linear internal residues of β(1→3)-Gal (11 mol.%) and β(1→6)-Gal (2.5 mol.%) (Duarte et al., 1971). The β(1→3)-Gal and β(1→3,6)-Gal constructed to main chain of galactan by randomly distributing manner, while terminal Gal and β(1→6)-Gal residues were existed in branches that substitutes at C-6 position by β(1→6) linkages. This backbone structure can be depicted form the result of Smith-degradation combining with methylation analysis between the degraded reserving polysaccharide and the prototypical one. It was because sugars of terminal D-Gal and β(1→6)-Gal in branches can be subsequently destroyed during the periodate oxidation (Diaz Segura et al., 1976).

Almost simultaneously, similar polysaccharide was isolated from egg masses of the snail *Ampullarius sp*., which belonged to fucogalactan on account of its Gal and Fuc residues containing. However, this fucogalactan exhibited appreciable difference in structure with the galactan isolated from albumen glands of the *Strophocheilus oblongus.* In brief, this fucogalactan was a highly branched polysaccharide and contained 27% of terminal-D-Gal, 29% of β(1→3,6)-Gal, 5% of β(1→3)-Gal, 26% of β(1→6)-Gal, 11% of β(1→2)-Gal and 2% of β(1→6)-Fuc residues in molar composition that were resulted from methylation analysis. D-Gal appeared as the non-reducing terminal groups and β(1→3,6)-Gal as the branching points, while

the residues in other linkages was used to extend the liner polysaccharide chain. Noticeably, it was the part of the internal residues of β(1→2)-Gal combining with the preponderance of β(1→6) over β(1→3) linkages that give special chain construction to this fucogalactan (Feijó et al., 1975). However, when Feijó et al. investigated once again on the galactans from snail eggs of *Ampullarius sp.*, they purified other different galactan preparations that deposited in the egg masses at various embryonic developing stages. Those galactans from snail egg masses had two components with sedimentation coefficients of 40 S and 10 S, and belonged to glucogalactan because of containing Gal and small proportion of Glc sugars in polymeric chain. Both 40 S and 10 S glucogalactans were highly branched polysaccharides with nonreducing ends containing α-D-Glc and β-D-Gal residues and with main chain comprising series of β(1→3,6)-Gal residues. In their backbone, one or more branches of Glc-α(1→4)-Gal-β(1→ was linked to C-6 position of Gal residues in backbone by β(1→6) linkages. Glucogalactans isolated from freshly masses of the snail egg being left for 10 and 15 days were structurally different from each other. With the embryonic development, it would occur the progressive diminution of 10 S component and the proportion of Glc in the polysaccharide, implying that each constituent was preferentially consumed by the snail embryos as an energy source (Feijó et al., 1982).

A new polysaccharide consisted of galactan sulfate with a β(1→3)-glycosidic linkage has been isolated from the marine clam species *Meretrix petechialis* (Fig. 4). The galactan sulfate was homogeneous in its composition since it only contained Gal sugar. The sulfate groups was mainly substituted at hydroxyls of C-6 or C-2 position of liner β(1→3)-Gal residues (Amornrut et al., 1999). It was not the only acidic galactan found in mollusks,

another galactan has been obtained with acetone precipitation from eggs extraction of the mollusk *Pomacea lineata* by Cruz et al.. However, sulfate and uronic acid has not been detected in molecules of the acid galactan of the snail egg by chemistry analysis. The structural investigation confirmed that the acidic galactan had a backbone containing β-D-Gal residues in a predominant content and presence of the β-D-GlcNAc in less proportion. In addition, pyruvate groups forming six-membered cyclic ketals were found in some Gal sugars and appeared as 4,6-O-(1'carboxy)-ethylidene-β-D-Gal residues with R configuration. It is the pyruvate groups that make the galactan becoming an acidic polysaccharide (Cruz et al., 2010).

3. 8. Other heterogeneous polysaccharides

Some polysaccharides rather than glucans or galatans were always found in *Mollusca* having intricate monosaccharide compositions and containing at least 3 monosaccharaides in their molecular chain. These compounds were attributed to heterogeneous polysaccharides with vague structure. Complicacies in monosaccharide composition made the heterogeneous polysaccharides building up a complex copolymer since their repeating units in molecular chain were indefinite. Structural elucidation in chain configuration and linkage pattern of these heterogeneous polysaccharides were in stagnating situation. Sometimes, complexity of their ingredients and difficulty in separation intensified the awkwardness too. However, relevant researches still promoted to more and more heterogeneous polysaccharides were found in species of *Mollusca* recently.

There was an appropriate instance on the mud snail of *Bullacta exarata* to elaborate it. Three sulfated polysaccharides, coded as BEMPA, BEMPB1, and BEMPB2, have been obtained

by extracting the mucilage secreted from the mud snail, and purifying with DEAE-cellulose ion-exchange and size-exclusion chromatography. Their average Mws were 23, 64 and 47.5 kDa, and sulfate content were 8.1, 12 and 9.7 wt.%, respectively. The structures analysis showed that the snail polysaccharides were heterogeneous and had distinct compositions in monosaccharide with each other. BEMPA, appearing with partially branches caused by presence of (1→3,4)-linked-Man, was composed of Rha, Glc, Man, Fuc and Ara in molar ratio of 2.2:1:4:1.1:1.8. BEMPB1 had branching points of Man sugars, and was made up of Rha, Glc, Man, Fuc, Gal and Ara in molar ratio of 3.3:1.3:2.8:1.2:1:7.3. BEMPB2 with partially branched structure was fabricated by Man, Fuc, Gal and Ara in molar ratio of 4.9:1.2:1:1.9. The sulfate substitutions of BEMPB1 were deduced to be at the C-3 site of (1→4)-linked-Man, and the sulfate groups of BEMPA and BEMPB2 were substituted at C-4 site of (1→3)-linked-Man (Zhang et al., 2013).

One purified polysaccharide named SVP-12 was obtained from viscus of one scallop *Patinopecten yessoensis* by proteolytic extraction, deproteination and chromatographies (Yan et al., 2009). SVP-12, with the Mw of about 170 kDa, appeared as a heterogeneous polysaccharide since it contains 72.1% of polysaccharide, 2.7% of protein, 12.6% of sulfate, and 8.5% of amino hexose. GC analysis indicated that SVP-12 comprised Rha, Fuc, Ara, Xyl, Man, Gal and Glc with the molar ratio of 1.7:2.5:4.1:5.6:1.5:4.9:1 (Yan et al., 2009). Yu et al. reported a similar heterogeneous polysaccharide from viscus of *Patinopecten yessoensis* and named it with SSVP-I. Polysaccharide of SSVP-I has 5.9 kDa of average Mw and contains Rha, Fuc, Ala, Xyl, Man, Gal and Glc residues in monosaccharides composition with molar ratio of 1:1.3:2.4:3:1.3:4.1:2.5 (Yu et al., 2009).

A fraction of water-soluble sulfated polysaccharide conjugate, termed AHP-2 (Zhu et al., 2011), was obtained from abalone (*Haliotis Discus Hannai* Ino) viscera by protease-assisted aqueous extraction followed by precipitation with ethanol and purification with gel filtration chromatography. AHP-2 is a heteroglycan appearing with an average Mw of about 11 kDa and consisting of Glc, Fuc, Xyl, Rha and Gal with molar ratio of 1:2:3.9:6.7:7.4. The backbone of AHP-2 consists of (1→3)-linked Rha and (1→3,6)-linked Gal, with Glc, Fuc, Xyl and Gal of different linkage types distributing in branched chains. Series of sulfate heterogeneous polysaccharide fraction (AGP31, AGP32, AGP33) have been isolated from the gonads of *Haliotis discus hannai* Ino with 37.8, 32.2 and 27.5 kDa of average Mw, respectively (Zhao et al., 2016b). All of them contained Man, Rha, GlcA, Glc, Gal, Xyl, Ara, and Fuc, and was represented as high similarity in monosaccharide profile. Obviously, their monosaccharide compositions were inconsistent with the ASGP polysaccharide mentioned above (Wang et al., 2015b). Heterogeneous polysaccharide AGP33 was selectively subjected to structural analysis after getting rid of branches and sulfate groups (Zhao et al., 2016a). It was found that the backbone moiety of AGP33 appeared to be mainly constituted with Man, Glc and Gal residues and constructed into five types of linkage, including α(1→3)-Glc, α(1→4)-Gal, and α(1→6)-Glc, α(1→6)-Man, and α(1→6)-Gal in molar ratio of 6.7:13.3:6.3:6:3.2. The α(1→4)-Gal and α(1→6)-Glc residues linked together as a disaccharide unit of Δα(1→4)-Gal-α(1→6)-Glc that distributed in main chain of AGP33. Meanwhile, the sulfating groups usually occurred at C-3 and C-4 positions of the monomer in chain (Zhao et al., 2016a).

4. Bioactivities and health benefits of mollusks polysaccharides

4. 1. Anticoagulant and antithrombin activities

Unfractionated Hep is the main anticoagulant drug used in cardiovascular surgery for the prevention of arterial or venous thrombosis. The worldwide consumption of Hep has been augmented to 100 tons/year, partially because of an increasing use of low Mw of Hep. However, the source of pharmaceutical Hep is very limited, because it is primarily obtained from porcine intestine. It was estimated that 20 million people suffered from thromboembolic diseases, and 200 million pigs were needed to meet this demand every year. Therefore, there is an urgent necessity to find alternative sources of Hep. Hep and/or the similar polysaccharide isolations with significant anticoagulant activity have been extensively described in mollusks (Table 4). Nineteen species from 14 families of bivalve mollusks contain HS/Hep like polysaccharides with anticoagulant activities ranging from 5 to 350 IU/mg. Many polysaccharide preparations isolated from those species of mollusks are directly identified to be Hep/HS compounds by structural analysis as mentioned above (Section 3.1). It was believed that the anticoagulant activity of Hep/HS is mainly mediated by antithrombin and heparin cofactor II, because the absence of these inhibitors drastically reduces anticoagulant activity of drugs. The anticoagulant activities of mollusk Heps have been evaluated determined by prolongation of APTT or prevention of the clotting of plasma. The antithrmbin activities of mollusks Hep-like polysaccharides were usually determined by the classical methods in binding of antithrombin or inhibition of the relevant protein factors (thrombin and Factor Xa). The assays were performed under using the standard Hep derived from mammalian as reference with 150 ~ 200 IU/mg of activities. The general results were depicted as below.

A large portion of Hep fractions isolated from two marine clam species *Anomalocardia brasiliana* and *Tivela mactroides* could bound with high affinity to immobilized antithrombin. Polymeric chain of such Hep fractions contained up to three binding sites to antithrombin and was observed with higher binding constants than those of commercial Hep at comparable Mw level (Pejler et al., 1987). Cesaretti et al. described a clam Hep from *Tapes phylippinarum* having the ATIII binding site due to the presence of large amounts of the specific oligosaccharide sequence with high sulfated content. The clam Hep possessed high anticoagulant activity in APTT of 347 IU/mg and anti-factor Xa activity of 317 IU/mg (Cesaretti et al., 2004). The unusual Hep fraction purified from the marine Italian bivalve mollusk *Callista chione* were test having 97 IU/mg of anticoagulant activities in APTT and 52 IU/mg of anti-factor Xa activity (Luppi et al., 2005a). One partially purified Hep fraction from fresh water mussel *Anodonta anodonta* showed a prominent anticoagulant activities of 137 IU/mg in APTT and 120 IU/mg for anti-Xa, which was substantial to commercial Hep in test (Volpi et al., 2005). Two Hep fractions purified from *Tridacna maxima* and *Perna viridis* were measured by factor Xa amidolytic assay and showed about 20.6 and 12.05 IU/mg of activities. However, their anticoagulant activities were significantly lower than that of commercial Hep from porcine mucosa with 120.5±23.5 IU/mg of anti-factor Xa activities (Arumugam et al., 2009a). At same conditions, the fractionated Hep-like polysaccharides from marine mollusks *Meretrix meretrix* and *Amussium pleuronectus* showed with 72 and 95 IU/mg of activities in APTT tests, respectively (Saravanan et al., 2010a; Saravanan et al., 2010b). Hep GAGs isolated from two clams of *Donax sp*. have been proved possessing potent anticoagulant activities when tested by preventions of the clotting of sheep plasma (Periyasamy et al., 2013; Vijayabaskar et al., 2012). One crude Hep fraction and its

purified products from *Meretrix casta* have demonstrated with 64.1 IU/mg and 35.3 IU/mg of APTT activities, respectively (Vidhyanandhini et al., 2014). HS-like preparation from mollusk *Nodipecten nodosus* has an anticoagulant activity of 36 IU/mg, 5-fold lower than standard Hep (180 IU/mg), as measured by the APTT assay. It also inhibits factor Xa and thrombin with IC_{50}=835 μg/ml and 9.3 mg/ml in antithrombin assay, respectively. Furthermore, a minor additional anticoagulant activity of this mollusk HS was found when antithrombin and heparin cofactor II are absent in evaluating system, indicting the involvement of serpin-independent mechanism. *In vivo* assays demonstrated that the *N. nodosus* HS inhibited thrombus growth in photochemically injured arteries at the dose of 1 mg/kg. No bleeding effect, factor X and IIa-mediated kallikrein activity, or toxic effect on fibroblast cells was induced by this HS at the antithrombotic (Gomes et al., 2010).

It is also paid attention to other types of polysaccharide differing with Hep/HS from mollusks on their anticoagulant potentials. DS isolated from the body of marine clam *Scapharca inaequivalvis* was calculated to possess a high heparin cofactor II activity (169.2 IU/mg) that was fairly similar to commercial DS purified from porcine tissue. Although the anticoagulant activity of this DS is somewhat lower than that of mammalian Hep, its venous antithrombotic activity appears to be significantly higher with its hemorrhagic side effects greatly reduced. This resulted to the clam DS becoming an interesting strategy to develop new therapeutic agents in prevention of thrombosis (Volpi et al., 2009). One novel GAG-like sulfated polysaccharide (AAP) from the pleopods of *Haliotis discus hannai* Ino prolonged both the APTT (22.5 IU/mg) and TT (72 IU/mg), but is invalid on PT by *in vitro* anticoagulant assays. AAP could be deemed to a Chn-like polysaccharide in the

anticoagulant potential and its anticoagulant effect was considered to affect the multi-coagulation, especially the last coagulant step of the thrombin-mediated fibrin formation. AAP had a significant inhibitory effect on thrombin activity when mediated by ATIII nearly the same level as the standard heparin (Li et al., 2011). Sulfated polysaccharide fractions AGP33 isolated from the gonads of *Haliotis discus hannai* Ino could similarly prolong APTT, TT and PT, as compared to a saline control solution, and exhibited with a dosage-dependent manner (Zhao et al., 2016a).

The difference in anticoagulant activity of the mollusks polysaccharides are mainly attributed to their different compositions, sulfate content and variable structure including sugar sequences, sulfate positions and Mw. Crude GAGs with more complex compositions have stronger activity than purified GAGs from *Marcia casta* attributing to the high content of sulfate (Vidhyanandhini et al., 2014). The increase of sulfate groups in polysaccharide can increase both their specific and non-specific binding to a wide range of thromboplastic proteins (Cesaretti et al., 2004). Polysaccharide with higher Mw demonstrated greater anticoagulant activity (Zhao et al., 2016a). Hep/HS GAGs have a more heterogeneous structure due to sulfated regions distributing along the chain (Arumugam et al., 2009a). The antithrombin-binding region of the Hep molecule has been identified as a specific pentasaccharide sequence that contains a unique 3-O-sulfated GlcN residue. This component is absent in heparin with low affinity for antithrombin and, hence, low anticoagulant activity (Luppi et al., 2005a; Pejler et al., 1987; Volpi et al., 2005). However, another sequence ΔIdA-GlcN3S6S occurred in mollusks Heps appeared not to be involved in the interaction with ATⅢ(Pejler et al., 1987)**.** Furthermore, other

studies have also indicated that the IdoA/GlcA ratio may also contribute significantly to the capacity of mollusks GAGs to inhibit HCII-mediated thrombin activity (Volpi et al., 2009).

4. 2. Antiatherogenic activities

GAGs from mollusks are more concerned in developing anti-atherosclerotic drugs recently, since it is clear that protection of GAGs on the endothelial cell may relate to the anti-atherosclerotic mechanism. Two types of GAG polysaccharides derived from scallop skirt and oyster were evaluated their potentials on protecting the damaged vascular endothelial cells from prevention or treatment aspects. The scallop skirt glycosaminoglycan (SS-GAG) reduce the damage of cultured human umbilical vein endothelial cells that injured by oxygen free radicals, oxidized low density lipoprotein, or regents of Fenton reaction (Wang et al., 2006; Zhang et al., 2006; Zhang et al., 2004a; Zhang et al., 2004b), and inhibit highly concentrate glucose to damage the vascular endothelial cells (ECV-304) (Wang et al., 2008a). Results exhibited the anti-atherosclerotic potentials of scallop skirt GAG on account of its apparent protecting activities to the injured endothelial cells. SS-GAG was also proved to have strong antiatherogenic effect due to its ability to inhibit the foam cell formation and increase glutathione peroxidase and NO expression, when it was tested in a model of porcine artery smooth muscle cells transforming into foam cells by incubation of high concentration of aoxidized low-density lipoprotein (Sun et al., 2005). At same time, Wang et al. have investigated the influences of oyster GAG extracts on the function of injured human vascular endothelial cells. Examination on the model of vascular endothelial cells injured by H_2O_2 proved that pretreatment with oyster GAG can enhance the

antioxidant capacity of human vascular endothelial cell, promote secretion of NO, and obviously have protection effect on injured human vascular endothelial cells (Wang et al., 2007). One Hep like GAG isolated from mollusk of *Donax cuneatus* could be a best alternative source for the development of hypocholesterolemia drugs. Once used in the treatment of hypocholesterolemia atherogenesis of male Wistar rat model, it could modulate the early lipid changes, and down-regulate activities of the key enzymes of atherosclerosis formation, maintain the normal function of liver and kidney (Pandian et al., 2009). All this study hinted that GAG kinds of polysaccharides from *Mollusca* may have important effect on preventing and curing cardiovascular disease, diabetes and arthrosclerosis.

4. 3. Antioxidant activities

Antioxidants can protect human body against damage by ROS, and thus they can prevent oxidative degeneration and may definitively have a positive effect on human health. Recently, there is a considerable interest in the food industry as well as pharmaceutical industry for the development of antioxidants from natural sources, including mollusks (Liu et al., 2008b). The polysaccharides prepared from mollusks were one of the useful candidates in search for effective, non-toxic substances with potent antioxidant activity (Yin et al., 2007; Zhao et al., 2010), because they could be served as free-radical inhibitors or scavengers, acting possibly as primary antioxidants (Zhu et al., 2010b).

Crude extracts or purified products of polysaccharide isolated from many species of mollusks, including *Patinopectem yessoensis*, *Sinonovacula constricta*, *Mactra chinensis* and *Corbicula fluminea*, have been proved exhibiting potential *in vitro* antioxidant effect.

Scavenging ability to hydroxyl free radicals of SVP polysaccharide isolated from viscus of scallop (*Patinopectem yessoensis)* was up to 84.75% at 6.5 mg/ml of concentration (Yin et al., 2007). Scallop gonad polysaccharide extract (SGP) also were examined to have reducing power and scavenging effect on DPPH and hydroxyl free radicals (Song et al., 2012). The crude polysaccharide product isolated from razor clam *Sinonovacula constricta* had scavenging effect on hydroxyl free radicals, and the scavenging hydroxyl free radical ability was increased up to 94.76% and scavenging superoxide anion radical ability was up to 83.08% (Zhao et al., 2010). Crude polysaccharides extracted from Chinese surf clam (*Mactra chinensis)* had strong antioxidant properties and the scavenging rates against superoxide anion and hydroxyl free radicals at 0.8 mg/ml were 86.49% and 49.31%, respectively (Chang et al., 2012). One GAG similar polysaccharide, CFPS-2, isolated from the fresh water clam *Corbicula fluminea,* exhibited strong antioxidant activities in a dose dependent manner (Liao et al., 2013). Moreover, as far as the emphasized developing potential is concerned, antioxidant activity of mussel polysaccharides was even investigated in aged mice. After oral administration with 450 mg/kg of mussel polysaccharide, the T-AOC level was significantly increased in serum and liver tissue of the pretreated aged mice, the contents of MDA and 8-ISO-PG were decreased, the enzyme activities of GSH-PX, T-SOD were increased and the contents of GSH were increased with significant differences in comparison with the control group, indicating mussel polysaccharides have stronger antioxidant effect on the aged mice (Li et al., 2015) .

4. 4. Immunomodulatory activities

Compounds of polysaccharide have been shown playing important effects on the immune systems by stimulating the immune response or regulating immune cells' function through multiple targets and passages, and have impact on disease progression and outcomes, such as microbial infection, serve inflammations, tumor progression and metastasis (Zhu et al., 2010a).

GAG type of polysaccharides isolated form mollusks usually are conjunct with some proteins that making them are antigenic and can firstly cause humoral immune responses when they entered into internal environment of human body (Karamanos et al., 1990). On other side, the purified polysaccharide moieties excluding proteins could improve both specific and non-specific cellular immune response. Scallop gonad polysaccharide (SGP) at the 0.2μg/ml could not only protect RAW264.7 cells from oxidative damage induced by n-butyl, but also promote lymphocyte proliferation and activate complement activity (Song et al., 2012).

The other polysaccharides dislike GAGs types mostly referring to water soluble glucans from mollusks, have also been proved to be more potent immune enhancing agents, such as HCLPS-1 from clam of *Hyriopsis cuming*ii Lea (Dai et al., 2009), MMPX-B2 from *Meretrix meretrix* Linnaeus (Li et al., 2014), SCP-1 from *Sinonovacula constricta* (Yuan et al., 2015), and oyster polysaccharide (O-P) (Li et al., 2013). These polysaccharides exert their immunostimulating effects mainly basing on macrophages modulation. HCLPS-1 not only could significantly promote Con-A, LPS-stimulated splenocytes proliferation in concentration-dependent manner *in vitro*, but also increase Con-A and LPS induced splenocytes proliferation in mice immunized with the sheep red blood cell, and it could remarkably promote DNFB-induced delayed-type hypersensitivity reactions as well (Dai et al., 2009). MMPX-B2

could stimulate the murine macrophages to release various cytokines for immune defense (Li et al., 2014). SCP-1 could significantly increase the viability of macrophages, enhance the capability of macrophage phagocytosis, increase the activity of acid phosphatase, and promote the production of nitric oxide, mouse TNF-α, IFN-γ, and IL-1β (Yuan et al., 2015). O-P could significantly increase the thymus index and spleen index, reinforce the phagocytosis ability of the macrophages and possess the immunologic enhancement role in mice infected by HSV-1 (Li et al., 2013).

4. 5. Antiviral activities

Sulfated polysaccharides and GAG like polysaccharides from mollusks possessed antiviral activities because of the sulfate containing and have huge potential in developing antiviral drugs or functional foods. A galactan sulfate purified from *Meretrix petechialis* has anti-HIV activity that exhibited with inhibition of syncytia formation. The fusion index and percentage fusion inhibition of the sulfated galactan were 0.34 and 56% at 200 μg/ml of concentration (Amornrut et al., 1999). Woo et al. further confirmed the potent anti-HIV activity of sulfated polysaccharide from *Meretrix petechialis* after repeating the test. In their test, the fusion index and percent fusion inhibition were 0.21, and 67.52 at 100μg/ml, respectively, and almost equivalent to that of dextran sulfate (Woo et al., 2001). The inhibitory effect of scallop skirt glycosaminoglycan (SS-GAG) on HSV-Ⅰin different time and concentration has been investigated (Yu et al., 2008). SS-GAG showed significant activity of protecting host cell and antiviral effect against HSV-Ⅰ *in vitro*. SS-GAG treatment could lessen the cytopathic effect induced by HSV-Ⅰand inhibit virus replication,

and protect the host cells effectively and heighten the cell activity as well. The antiviral effect increased along with the prolongation of SS-GAG used, the highest efficiency of resistance could reach of 88.64% under the determined doses (Yu et al., 2008). Oyster polysaccharides (O-P) had obvious restrain effect on infection of HBV to HepG2.2.15 cells by depressing duplication of virus DNA and decreasing the secretion of HBsAg and HBeAg. The IC_{50} of O-P on HBsAg and HBeAg were 294 ug/ml and 168 ug/ml and the therapeutic index was 17 and 29.8, respectively (Fan et al., 2011). The antiviral activity of SCP polysaccharides from scallop has been evaluated on duck Hepatitis B virus replication in ducklings' *in vivo* examination. It was found that the optic density value of Hepatitis B virus DNA in ducks serum were obviously decreased after giving medication with scallop polysaccharides at 150 and 300 mg/kg of dosage, indicating that scallop polysaccharides have significant inhibitory effects on HBV infection (Fan et al., 2012).

4. 6. Antitumor activities

Several studies have reported that polysaccharides from mollusks have anti-proliferative activity in cancer cell lines *in vitro,* as well as inhibitory activity of tumor growth in mice. The anti-tumor effects are more related with the sulfated polysaccharide, especially the GAG like polysaccharides from mollusks, since they could play important roles in physiological and pathological conditions, let alone the antitumor behavior. One Hep isolated from *Perna viridis* showed higher anti-proliferative activity when it was test with pulmonary artery smooth muscle cells (Arumugam et al., 2009b). Fuc-rich GAG fractions isolated from Squid ink exhibited strong antitumor activity against Meth-A fibrosarcoma in BALB/c mice following intraperitoneal administration (Matsue et al., 1997). Similar polysaccharide, named as SIP, isolated from the ink

of cuttlefish, *Sepiella maindroni*, could significantly reduce the frequency of micro nucleated cells in polychromatic erythrocytes and reticulocytes induced by cyclophosphamide in tumor-bearing mice, revealing its strong antimutagenic activity (Liu et al., 2008a). One purer fraction of SIP polysaccharide called SIP-SII could suppress invasion and migration of carcinoma cells via inhibition of MMP-2 proteolytic activity although it weakly inhibited tumor cell growth without cytotoxicity *in vitro* assay (Wang et al., 2008b). CFPS-2, a GAG similar polysaccharide, isolated from fresh water clam *Corbicula fluminea*, also exhibited significant inhibitory effects on growth of human gastric cancer cells (SGC7901) and human ovarian carcinoma cells (SKOV3 and A2780) (Liao et al., 2013). The crude GAGs isolated from different mollusks of whelks and cockles demonstrated variable selective anti-cancer activities against many cancer cell lines including breast (MDANQ01 and MDA468), leukemia (MOLT-4 and K562) and ovarian (HeLa) cancer (Ogundipe, 2015). Ogundipe et al. found that GAGs purified from mollusks cloud bring significant perturbations in the cancer cell cycle, made cell cycle arrests at different stages, and synchronously cause significant apoptosis of tumor cells. They further revealed that structural composition of mollusk GAG, especially sulfation levels or patterns, may be partly responsible for their anti-cancer activities as it can alter its binding to some growth factors, which is essential for tumor cell proliferation (Ogundipe, 2015).

The polysaccharide purified from edible mussel has inhibitory effect on HL-60 tumor cells *in vitro*, indicating it is a sulfated polysaccharide in chemical nature (Li et al., 2008). Sulfated mannan extracts from the mucilage of mud snail of *Bullacta exarata* exhibited high inhibitory effects on growth of B-16 melanoma cells, and IC_{50} were 31.1μg/ml (Zhang et

al., 2013). Sulfating modification for Mussel polysaccharide could increase its inhibiting activity against tumor cells (Du et al., 2014). The above investigations indicated that sulfate groups are crucial to the antitumor activities of polysaccharide from mollusks. However, water soluble glucan compounds PE isolated from *Ruditapes philippinarum* showed significant tumoricidal activity against human hepatoma SMMC-7721 cells and strong antitumor activity against solid tumor Sarcoma 180 in a dose-dependent manner, and demonstrated stimulating effect on murine lymphocyte proliferation induced by Co-A as well. The PE polysaccharide was nonsulfated polysaccharide, it is likely that presence of bound protein, compositional glucan and moderate Mw endow its antitumor activities (Zhang et al., 2008).

4. 7. Other bioactivities

In order to amplify the application fields, other bioactivities were also preliminarily investigated for assessing the polysaccharide compounds from mollusks, including neuroregulatory activities of squid cartilage CS-E, hepatoprotective effects of mussel polysaccharide and oyster polysaccharide, gastroenteric improving function of polysaccharides from abalone viscera or abalone gonad, and antibacterial abilities of polysaccharide from *Mactra chinensis.*

CS-E from squid cartilage could exhibit neuroregulatory activities, which are expressed through molecular interactions with specific proteins, including heparin cofactor II, selectins, CD-44, chemokines, and the heparin-binding growth factor midkine (Kinoshita et al., 2001). Both mussel polysaccharide and oysters polysaccharides (derived from *Ostrea gigas*) had a protective role in acute alcoholic liver injury in mice (Hou et al., 2014; Li et al., 2009). Oral

administrations with mussel polysaccharides or oysters polysaccharides could normalize the over amount of ALT, AST and TG in serum, and enhance the content of GSH, MDA in liver homogenate, and ameliorate the damages that induced by uptake of alcohol to mice. AHP-2 polysaccharide obtained from abalone viscera and AGP series of polysaccharide fractioned from abalone gonad even were considered to improve gastroenteric function since they were found to can increase CCK release in CCK-secreting STC-1 cells (Zhao et al., 2016b; Zhu et al., 2011). Further study indicated that Ca^{2+}/CaM/CaMK, cAMP/PKA and MAPK pathways were all involved in AGP-induced CCK secretion (Zhao et al., 2016b). Furthermore, polysaccharide isolated from Chinese surf clam (*Mactra chinensis*) was proved to have some antibacterial abilities and showed stronger activity against Gram-positive bacteria than Gram-negative bacteria (Chang et al., 2012).

5. Summery and perspectives

Mollusca bioresource is a treasure deposit in the world and definitely deserve to explore and utilize due to its huge output and plentiful species. Many mollusks contained abundant carbohydrates as valuable chemicals with high molecular pattern since they were synthesized in tissues to fulfill many biofunctions (Medeiros et al., 1998). Polysaccharide compounds in mollusks could vary in content, category, and composition with species, living environment, developmental stages, and tissue location, depending on the physiological requirement of themselves (Karanova, 2006; Medeiros et al., 1998; Pandian et al., 2008). Considerable studies have been attempted to investigate the structural characteristics and functional features of polysaccharide compounds from cephalopods,

gastropods and bivalves (Zhang, 2009). However, species number has been involved in polysaccharide research is relatively low compared to the total species in *Mollusca*. Much more species should be pay attention on this topic than that concerned in this review and more and more polysaccharides with novel structure and strong activities would be thereby found further.

It was highlighted that most types of GAG polysaccharides, including Hep/HS, CS/ DS, and HA, combining with other typical polysaccharides such as GAG analogues, gulcans, galactosans and various hybrids of glycan with or without sulfates, could be isolated from *Mollusca* animals. Polysaccharides isolated from mollusks appeared with high degrees of heterogeneity in primary structure with regards to monosaccharide composition, glucosidic bond linkages, repeating unit construction, sulfation and Mw. On account of the structural diversity and heterogeneity, difficulties in purification, separation and structural determination are challenging in developing the mollusks polysaccharide as nature products. Synthesis of a standardized commercial product based on mollusks polysaccharide constituents is expected to be a significant endeavor. Sometimes, mixed or crude polysaccharide was an alternative objective material if it can be proved to be active.

Recent studies prove that mollusks polysaccharide perform vital medical potentials including anticoagulant, antiatherogenic, antioxidant, immunomodulatory, antivirus and antitumor activities, and so on. By-products of mollusks processing with bioactive polysaccharide can easily be utilized to yield functional ingredients or even therapeutic agents. Considering the valuable biological functions and beneficial effects of mollusks derived polysaccharides, the possibility of designing new medicines and functional products to support

treating or regulating related diseases is promising. However, to date, most biological activities on mollusks derived polysaccharides have been superficially observed *in vitro* or in mouse models. It is necessary to evaluate and confirm these activities with more comprehensive methods, such as testing in large animals or human levels. Based on this consideration, paralleled investigations should be applied to correlations between activity and structure of mollusks polysaccharides. More attention paying to members of GAGs and sulfated glycans, especially on the Hep/Hs and sulfate glucans derived from marine snails and clams would benefit to drugs development for cardiocerebral vascular system and immune system.

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Table 1. The statistical *Mollusca* species involved in research of carbohydrate polymers and their taxonomy in class, order and family

Class	Order	Family	Genus and Species	References
Bivalvia	*Adapedonta*	*Pharidae*	*Sinonovacula constricta*	(Luan et al., 2015; Yuan et al., 2015; Zhao et al., 2010)
Bivalvia	*Adapedonta*	*Solecurtidae*	*Tagelus gibbus*	(Medeiros et al., 1998; Souza et al., 1985)
Bivalvia	*Arcoida*	*Arcidae*	*Anadara granosa*	(Teanchai et al., 2016)
Bivalvia	*Arcoida*	*Arcidae*	*Barbatia obliquata*	(Rupavathi et al., 1984)
Bivalvia	*Arcoida*	*Arcidae*	*Scapharca inaequivalvis*	(Volpi et al., 2009)
Bivalvia	*Mytiloida*	*Mytilidae*	*Mytilus galloprovincialis*	(Celik et al., 2014; Volpi et al., 2003)
Bivalvia	*Mytiloida*	*Mytilidae*	*Mytilus edulis*	(Grienke et al., 2014; Zhou et al., 2013)
Bivalvia	*Mytiloida*	*Mytilidae*	*Other Mytilus sp.*	(Du et al., 2014; Jung, 1998; Li et al., 2008, 2009; Li et al., 2015; Liu et al., 2008b; Zhang, 2009)
Bivalvia	*Mytiloida*	*Mytilidae*	*Perna viridis*	(Arumugam et al., 2009a; Arumugam et al., 2009b)
Bivalvia	*Mytiloida*	*Mytilidae*	*Perna canaliculus*	(Grienke et al., 2014)
Bivalvia	*Ostreoida*	*Ostreidae*	*Ostrea gigas*	(Hou et al., 2014; Ponis et al., 2003)
Bivalvia	*Ostreoida*	*Ostreidae*	*Ostrea edulis*	(Celik et al., 2014)
Bivalvia	*Ostreoida*	*Ostreidae*	*other oysters*	(Wang et al., 2007; Wang et al., 2006)
Bivalvia	*Ostreoida*	*Pectinidae*	*Amussium pleuronectus*	(Saravanan et al., 2010a)
Bivalvia	*Ostreoida*	*Pectinidae*	*Argopecten irradians*	(Wang et al., 1994; Wang et al., 1995; Wang et al., 1996)
Bivalvia	*Ostreoida*	*Pectinidae*	*Chlamys farreri*	(Li et al., 2004)
Bivalvia	*Ostreoida*	*Pectinidae*	*Nodipecten nodosus*	(Gomes et al., 2010)

Bivalvia	*Ostreoida*	*Pectinidae*	*Patinopecten yessoensis*	(Cui et al., 2012; Molchanova et al., 1992; Yan et al., 2009; Yin et al., 2007; Yu et al., 2009)
Bivalvia	*Ostreoida*	*Pectinidae*	*Pecten maximus*	(Latire et al., 2014)
Bivalvia	*Pterioida*	*Pteriidae*	*Pinctada fucata*	(Wada et al., 1971)
Bivalvia	*Unionoida*	*Unionidae*	*Anodonta anodonta*	(Volpi et al., 2005)
Bivalvia	*Unionoida*	*Unionidae*	*Anodonta californiensis*	(Hovingh et al., 1993)
Bivalvia	*Unionoida*	*Unionidae*	*Anodonta cygnea*	(Lopes-Lima et al., 2005)
Bivalvia	*Unionoida*	*Unionidae*	*Hyriopsis cumingii*	(Dai et al., 2009)
Bivalvia	*Unionoida*	*Unionidae*	*Unio pictorum*	(Marie et al., 2008)
Bivalvia	*Veneroida*	*Dreissenidae*	*Dreissena polymorpha*	(Fallis et al., 2010)
Bivalvia	*Veneroida*	*Donacidae*	*Donax cuneatus*	(Pandian et al., 2009; Vijayabaskar et al., 2009; Vijayabaskar et al., 2012)
Bivalvia	*Veneroida*	*Donacidae*	*Donax faba*	(Periyasamy et al., 2013)
Bivalvia	*Veneroida*	*Cyrenidae*	*Corbicula fluminea*	(Liao et al., 2013)
Bivalvia	*Veneroida*	*Mactridae*	*Mactra chinensis*	(Chang et al., 2012)
Bivalvia	*Veneroida*	*Mactridae*	*Mactra veneriformis*	(Wang et al., 2011a; Wang et al., 2013; Wang et al., 2015b; Wang et al., 2011b)
Bivalvia	*Veneroida*	*Tridacnidae*	*Tridacna maxima*	(Arumugam et al., 2009a; Arumugam et al., 2009b)
Bivalvia	*Veneroida*	*Veneridae*	*Anomalocardia brasiliana*	(Dietrich et al., 1990; Medeiros et al., 1998; Pejler et al., 1987; Souza et al., 1985)
Bivalvia	*Veneroida*	*Veneridae*	*Callista chione*	(Luppi et al., 2005)
Bivalvia	*Veneroida*	*Veneridae*	*Katelysia opima*	(Somasundaram et al., 2007; Vijayabaskar et al., 2008)
Bivalvia	*Veneroida*	*Veneridae*	*Marcia opima*	(Pandian et al., 2008)
Bivalvia	*Veneroida*	*Veneridae*	*Merceneria mercenaria*	(Ademolu et al., 2015; Hillman, 1968, 1969; Ulrich et al., 2001)

Bivalvia	*Veneroida*	*Veneridae*	*Meretrix petechialis*	(Amornrut et al., 1999; Woo et al., 2001)
Bivalvia	*Veneroida*	*Veneridae*	*Meretrix lusoria*	(Ademolu et al., 2015)
Bivalvia	*Veneroida*	*Veneridae*	*Meretrix meretrix*	(Li et al., 2014; Saravanan et al., 2010b)
Bivalvia	*Veneroida*	*Veneridae*	*Meretrix casta*	(Vidhyanandhini et al., 2014)
Bivalvia	*Veneroida*	*Veneridae*	*Ruditapes decussatus*	(Anacleto et al., 2014)
Bivalvia	*Veneroida*	*Veneridae*	*Ruditapes philippinarum*	(Anacleto et al., 2014; Liu et al., 2013; Zhang et al., 2008)
Bivalvia	*Veneroida*	*Veneridae*	*Tivela mactroides*	(Dietrich et al., 1990; Pejler et al., 1987)
Gastropoda	*Architaenioglossa*	*Ampullariidae*	*Pomacea lineata*	(Cruz et al., 2010)
Gastropoda	*Architaenioglossa*	*Ampullariidae*	*Pomacea sp.*	(Jeronimo et al., 1989; Souza et al., 1985)
Gastropoda	*Architaenioglossa*	*Ampullariidae*	*Ampullarius sp.*	(Feijó et al., 1975; Feijó et al., 1982)
Gastropoda	*Cephalaspidea*	*Haminoeidae*	*Bullacta exarata*	(Zhang et al., 2013)
Gastropoda	*Basommatophora*	*Planorbidae*	*Planorbarius corneus*	(Volpi et al., 2007)
Gastropoda	*Basommatophora*	*Planorbidae*	*Helisoma duryi*	(Morishita et al., 2009)
Gastropoda	*Basommatophora*	*Lymnaeidae*	*Lymnaea stagnalis*	(Ballance et al., 2004; Karanova, 2006)
Gastropoda	*Stylommatophora*	*Achatinidae*	*Archachatina marginata*	(Ademolu et al., 2015)
Gastropoda	*Stylommatophora*	*Achatinidae*	*Achatina achatina*	(Ademolu et al., 2015)
Gastropoda	*Stylommatophora*	*Achatinidae*	*Achatina fulica*	(Ademolu et al., 2015; Chi et al., 2006; Jeong et al., 2001; Kim et al., 1996; Kim et al., 1998)
Gastropoda	*Stylommatophora*	*Strophocheilidae*	*Strophocheilus oblongus*	(Diaz Segura et al., 1976; Duarte et al., 1971)
Gastropoda	*Vetigastropoda*	*Haliotidae*	*Haliotis discus*	(Li et al., 2011; Sun et al., 2010; Wang et al., 2014; Wang et al., 2015a; Weiss et al., 2002; Zhao et al., 2016a; Zhao et al., 2016b; Zhu et al., 2010a; Zhu et al., 2011; Zhu et al.,

				2010b)
Gastropoda	*Mesogastropoda*	*Littorinidae*	*Littorina littorea*	(Ademolu et al., 2015)
Gastropoda	*Neogastropoda*	*Buccinidae*	*Volutharpa ampullacea perryi*	(Wang et al., 2015a)
Gastropoda	*Neogastropoda*	*Busyconidae*	*Busycon caniculatum*	(Lash et al., 1960)
Gastropoda	*Neogastropoda*	*Marginellidae*	*Volvarina rubella*	(Eckmair et al., 2016)
Cephalopoda	*Teuthida*	*Sepiidae*	*Sepiella maindroni*	(Liu et al., 2008a; Wang et al., 2008)
Cephalopoda	*Teuthida*	*Sepiidae*	*Sepia officinalis*	(Karamanos et al., 1991)
Cephalopoda	*Teuthida*	*Loliginidae*	*Loligo sanpaulensis*	(Lavall et al., 2007)
Cephalopoda	*Teuthida*	*Loliginidae*	*Loligo plei*	(Lavall et al., 2007)
Cephalopoda	*Teuthida*	*Loliginidae*	*Loligo lessoniana*	(Chandumpai et al., 2004)
Cephalopoda	*Teuthida*	*Loliginidae*	*Loligo formosana*	(Chandumpai et al., 2004)
Cephalopoda	*Teuthida*	*Loliginidae*	*Loligo vulgaris*	(Ianiro et al., 2014)
Cephalopoda	*Teuthida*	*Loliginidae*	*Loligo chenisis*	(Cuong et al., 2016)
Cephalopoda	*Teuthida*	*Ommastrephidae*	*Ommastrephes bartrami*	(Chen et al., 2008)
Cephalopoda	*Teuthida*	*Ommastrephidae*	*Dosidicus gigas*	(Jung, 2013; Jung et al., 2011; Jung et al., 2014; Youn et al., 2013)
Cephalopoda	*Teuthida*	*Ommastrephidae*	*Illex argentines*	(Cortizo et al., 2008)
Cephalopoda	*Teuthida*	*Ommastrephidae*	*some other squids*	(Fongmoon et al., 2007; Habuchi et al., 1977; Junichi et al., 2009; Karamanos et al., 1990; Karamanos et al., 1988; Kinoshita et al., 2004; Kinoshita et al., 1997; Kinoshita et al., 2001; Matsue et al., 1997; Shetty et al., 2009; Takaya et al., 1994; Vynios et al., 1990)
Cephalopoda	*Sepiida*	*Octopodidae*	*Enteroctopus dofleini*	(Higashi et al., 2015)
Cephalopoda	*Sepiida*	*Octopodidae*	*other octopus*	(Zhang et al., 1997)

Table 2. Structural characterizations of some typical Hep/HS GAGs from mollusks

Mollusks origins	Type	Mw (kDa)	Disaccharide compositions	Intra and Inter linkages of the disaccharide	Other structural information	Reference
Tapes phylippinarum	Hep	10.7	ΔIdoA2S-GlcN2S6S (80.6%); ΔGlcA-GlcN2S6S (4.0%); ΔIdoA2S-GlcN2S (6.0%); ΔIdoA-GlcNAc6S (1.8%); ΔGlcA-GlcN2S3S6S (2.3%); ΔIdoA-GlcN2S6S (0.2%).	α(1→4),α(1→4); α(1→4),β(1→4); α(1→4),α(1→4); α(1→4),α(1→4); α(1→4),β(1→4); α(1→4),α(1→4).	Similar to bovine Hep; More sulfated groups due to containing more ΔIdoA2S-GlcN2S6S and significant ΔGlcA-GlcN2S3S6S.	(Cesaretti et al., 2004)
Anodonta anodonta	Hep	Not reported	ΔIdoA2S-GlcN2S6S (77.5%); ΔGlcA-GlcN2S6S (4.7%); ΔIdoA2S-GlcN2S (8.6%); ΔIdoA-GlcNAc6S (1.3%); ΔGlcA-GlcN2S3S6S (2.9%).	α(1→4),α(1→4); α(1→4),β(1→4); α(1→4),α(1→4); α(1→4),α(1→4); α(1→4),β(1→4).	Similar to bovine Hep; Presence of Hep with two components, one having high molecular mass and degree of sulfation and the other possessing lower molecular mass and sulfate group amount.	(Volpi et al., 2005)
Anomalocardia brasiliana and *Tivela mactroides*	Hep	22.5	ΔGlcA-GlcN6S (18.0~35.0%); ΔIdoA2S-GlcN (5.3~9.8%); ΔIdoA2S-GlcN (14.0~40.0%);	α(1→4),β(1→4); α(1→4),α(1→4); α(1→4),α(1→4); α(1→4),β(1→4); α(1→4),α(1→4); α(1→4),β(1→4);	Containing higher amounts of 3-O-sulfated disaccharide; Presence with a novel sequences of ΔGlcNS-IdA-GlcNS3S6S.	(Pejler et al., 1987)

			ΔGlcA-GlcN3S (3.5~16.0%); ΔIdoA2S-GlcN6S (17.0~34.0%); ΔGlcA-GlcN3S6S (0.6~12.0%); ΔIdoA-GlcN3S6S (0.7~3.5%).	α(1→4),α(1→4).		
Callista chione	Hep	10.9	ΔIdoA2S-GlcN2S6S (40.6%); ΔGlcA-GlcN2S6S (11.4%); ΔIdoA2S-GlcN2S (15.3%); ΔIdoA-GlcNAc6S (2.1%); ΔGlcA-GlcN2S3S6S (1.4); ΔIdoA-GlcN2S6S (11.8%).	α(1→4),α(1→4); α(1→4),β(1→4); α(1→4),α(1→4); α(1→4),α(1→4); α(1→4),β(1→4); α(1→4),α(1→4).	Presence of low amounts of the trisulfated disaccharide and a significant increase of the disaccharides with non-sulfated IdoA or GlcA	(Luppi et al., 2005)
Pomacea sp.	HS	33.0~89.0	ΔGlcA-GlcN2S6S (18.2~44.7%); ΔGlcA-GlcN2S (7.6~35.4%); ΔGlcA-GalNAc6S (12.8~41.9%); ΔGlcA-GalNAc (12.1~35.2%).	β(1→4), α(1→4); β(1→4), α(1→4); β(1→4), α(1→4); β(1→4), α(1→4).	Similar to the HS from Beef pancreas; Mw and disaccharide compositions varied at different embryonic stages.	(Jeronimo et al., 1989)
Anomantidae sp.	HS	Not reported	ΔGlcA-GlcN2S6S; ΔGlcA-GlcN2S; ΔGlcA-GlcNAc6S; ΔGlcA-GlcNAc.	β(1→4), α(1→4); β(1→4), α(1→4); β(1→4),	Like the mammalian HS; GlcNS6S as the non-reducing end while ΔGlcA-GlcNAc6S as the reducing end.	(Ferreira et al., 1993)

				α(1→4).		
Anodonta californiensis	HS	27.0	ΔGlcA-GlcNAc; ΔGlcA-GlcN.	β(1→4), α(1→4); β(1→4), α(1→4).	More mono-sulfated GlcA appeared as GLcA2S or GlcA3S; Having insignificant amounts disaccharide composed with GlcA2S3S; Major with nonsulfated GlcNAc containing disaccharides and minor with GlcN containing disaccharide; Sugar of GlcN in disaccharide units are mostly sulfated at O-2 and/or O-6 position.	(Gomes et al., 2010)
Achatina fulica	acharan sulfate	29.0	ΔIdoA2S-GlcNAc; ΔIdoA-GlcNAc.	α(1→4), α(1→4); α(1→4), α(1→4).	Have a saccharide backbone comprised of an equal amount of IdoA and GlcNAc; Major in ΔIdoA2S-GlcNAc disaccharide and minor in ΔIdoA-GlcNAc sequences in chain.	(Chi et al., 2006; Kim et al., 1996; Kim et al., 1998)

Table 3. Structural characterizations of some typical Chn GAGs from mollusks

Mollusks origins	Type	Mw (kDa)	Disaccharide compositions	Intra and Inter linkages of the disaccharide	Other structural information	Reference
Squid cornea of *Sepia officinalis*	CS	72.5	ΔGlcA-GalNAc4S (52%); ΔGlcA-GalNAc4S6S (28%); ΔGlcA-GalNAc (11%); ΔGlcA-GalNAc6S (9%).	β(1→3),β(1→4); β(1→3),β(1→4); β(1→3),β(1→4); β(1→3),β(1→4).	Over-sulfated CS; Presence with some neutral sugar of Glu, Gal and Man in molecule.	(Karamanos et al., 1991)
Squid cornea of *Sepia officinalis*	CS	72.0	ΔGlcA-GalNAc4S (49%); ΔGlcA-GalNAc (30%); ΔGlcA-GalNAc6S (20%).	β(1→3),β(1→4); β(1→3),β(1→4); β(1→3),β(1→4).	Normal sulfated CS Containing trance ΔGlcA-GalNAc4S6S and low amount of neutral sugar.	(Karamanos et al., 1991)
Squid liver integument	CS	79.6	ΔGlcA-GalNAc4S (41.8%); ΔGlcA-GalNAc4S6S (21.6%); ΔGlcA-GalNAc6S (14.9%); ΔGlcA-GalNAc (13.9%); ΔGlcA3S-GalNAc4S (5%); ΔGlcA3S-GalNAc4S6S (2.8%).	β(1→3),β(1→4); β(1→3),β(1→4); β(1→3),β(1→4); β(1→3),β(1→4); β(1→3),β(1→4).	Presence with significant amounts of GlcA3S-containing disaccharides.	(Shetty et al., 2009)
Squid cartilage	CS	Not reported	ΔGlcA-GalNAc4S; ΔGlcA-GalNAc4S6S; ΔGlcA-GalNAc6S; ΔGlcA-GalNAc;	β(1→3),β(1→4); β(1→3),β(1→4); β(1→3),β(1→4); β(1→3),β(1→4);	Polytropes in disaccharide compositions depending on its origins;	(Habuchi et al., 1977; Kinoshita et al., 2004;

			ΔGlcA3S-GalNAc4S; ΔGlcA3S-GalNAc4S6S.	β(1→3),β(1→4); β(1→3),β(1→4).	Abundance with E series of disaccharide; Presence with significant amounts of GlcA3S-containing disaccharides; Some with Glu branches.	Kinoshita et al., 1997; Vynios et al., 1990)
Squid ink	Chn	50.0~80.0	ΔGlcA-Fuc.	β(1→4),α(1→3)	Non sulfated Chn; Equimolar ratio of Fuc, GlcA, and GalNAc; GalNAc as branches linked to Fuc by α(1→3) bonds.	(Matsue et al., 1997)
Squid ink sac of *Ommastrephes bartrami*.	Chn	48.0	ΔGlcA-Fuc.	β(1→4),α(1→3)	Non sulfated Chn; Equimolar ratio of Fuc, GlcA, and GalNAc; GalNAc as branches linked to Fuc by α(1→3) bonds.	(Chen et al., 2008)
Eggs of *Pomacea sp.*	CS	20.0~120.0	ΔGlcA-GalNAc4S (38.1~77.3%); ΔGlcA-GalNAc (22.1~61.9%).	β(1→3),β(1→4); β(1→3),β(1→4).	Molecular weights and disaccharides varied at different embryonic stages.	(Jeronimo et al., 1989)
Anodonta anodonta	CS	Not reported	ΔGlcA-GalNAc4S (48.0%); ΔGlcA-GalNAc6S (28.0%) ΔGlcA-GalNAc (26.0%)	β(1→3),β(1→4); β(1→3),β(1→4); β(1→3),β(1→4)	Belonging to low sulfated Chn	(Volpi et al., 2005)
Scapharca inaequivalvis	DS	27.0	ΔIdoA2S-GalNAc4S (39.0%) ΔIdoA-GalNAc4S (35.1%);	β(1→3),β(1→4); β(1→3),β(1→4); β(1→3),β(1→4); β(1→3),β(1→4).	A peculiar structure compared with common DS	(Volpi et al., 2009)

	ΔGlcA-GalNAc (20.0%); ΔGlcA-GalNAc6S (4.4%).

Table 4 Summary of anticoagulant activities of the typical polysaccharide samples purified from mollusks

Mollusks origins	Polysaccharide types	Methods of Assay	Anticoagulant activity	Reference
Tivela mactroide	A series of Hep fractions.	Anti-Xa Anti- IIa	2~185 IU/mg 69~315 IU/mg	(Pejler et al., 1987)
Anomdocardia brasiliana	A series of Hep fractions.	Anti-Xa Anti- IIa	0~238 IU/mg 33~220 IU/mg	(Pejler et al., 1987)
Tapes phylippinarum	Hep	APTT Anti-Xa	347 IU/mg 317 IU/mg	(Cesaretti et al., 2004)
Callista chione	Hep	APTT Anti-Xa	97 IU/mg 52 IU/mg	(Luppi et al., 2005)
Anodonta anodonta	Hep	APTT Anti-Xa	137 IU/mg 120 IU/mg	(Volpi et al., 2005)
Katelysia opima	Hep	APTT	160 IU/mg	(Vijayabaskar et al., 2008)
Tridacna maxima	Hep	Anti-Xa	20.6 IU/mg	(Arumugam et al., 2009a)
Perna viridis	Hep	Anti-Xa	12.05 IU/mg	(Arumugam et al., 2009a)
Amussium pleuronectus	Hep	APTT	95 IU/mg	(Saravanan et al., 2010a)
Meretrix meretrix	Hep	APTT	72 IU/mg	(Saravanan et al., 2010b)
Donax cuneatus	Three Hep fractions	Preventing the clotting of sheep plasma;	158~175 IU/mg	(Vijayabaskar et al., 2012)

		Anti-Xa	27.5~58.5 IU/mg	
Donax faba	Hep	Preventing the clotting of sheep plasma	114 IU/mg	(Periyasamy et al., 2013)
Meretrix casta	Hep	APTT	35.2 IU/mg	(Vidhyanandhini et al., 2014)
Scapharca inaequivalvis	DS	HCII-mediated thrombin (factor IIa) inhibition	169.2 IU/mg	(Volpi et al., 2009)
Nodipecten nodosus	HS	APTT Anti-Xa Anti- IIa	38.3 IU/mg 36 IU/mg 16 IU/mg	(Gomes et al., 2010)
Haliotis discus hannai Ino	GAG similar (APP)	APTT PT	22.5 IU/mg 72 IU/mg	(Li et al., 2011)
Haliotis discus hannai Ino	GAG similar (AGP)	APTT TT PT	Effect with dose dependence	(Zhao et al., 2016a)

Figure captions

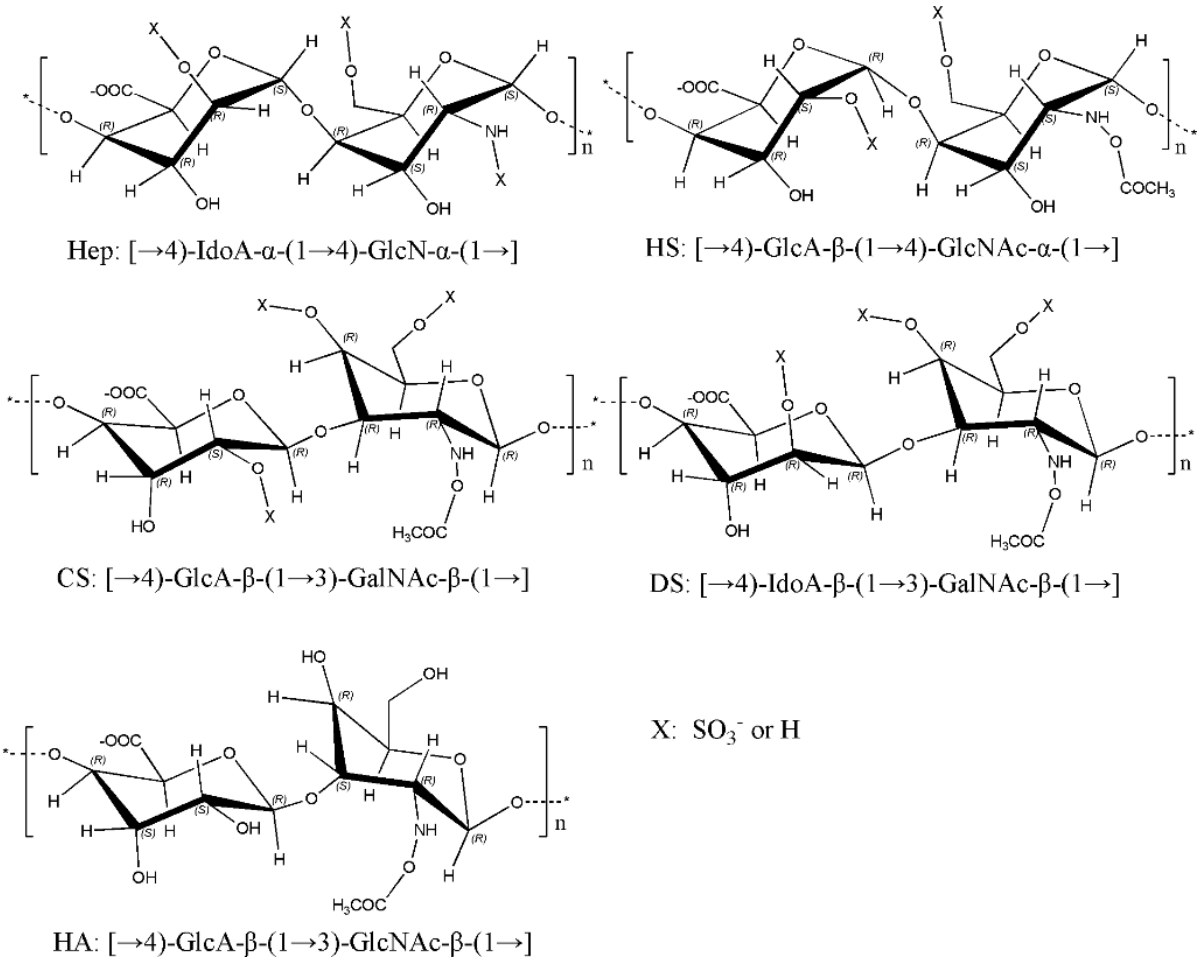

Fig. 1. The chemical structures in visualization their repeating disaccharide unit for the classical Hep, HS, CS, DS and HA molecules.

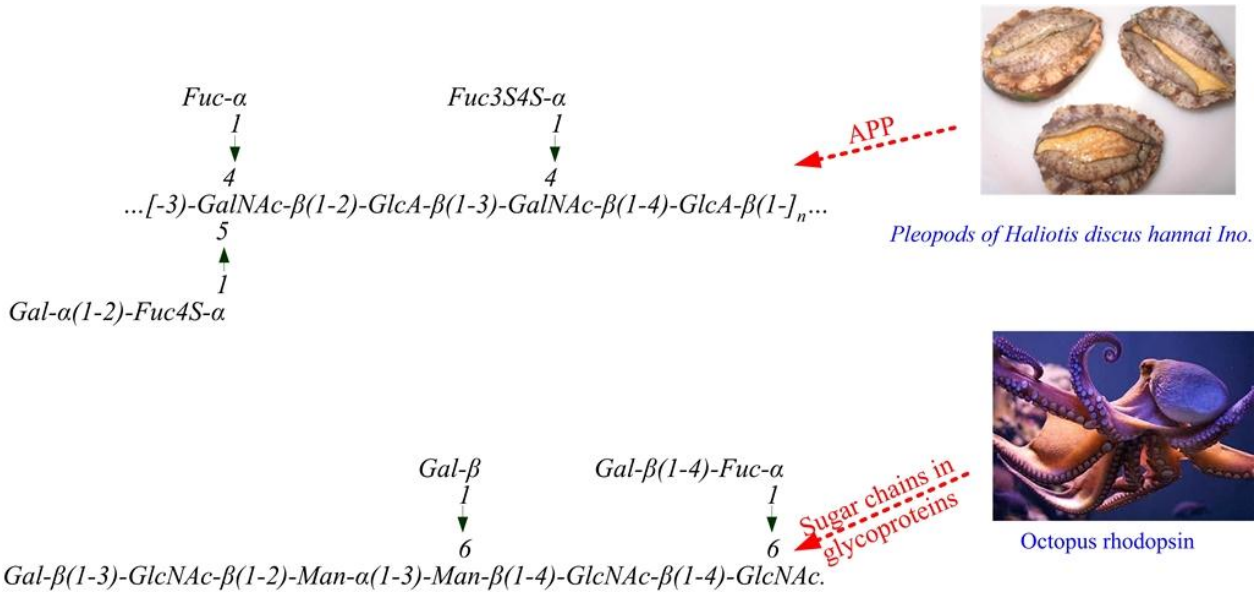

Fig. 2. The structural drawings of two unusual GAG similar polysaccharides purified from abalone and octopus.

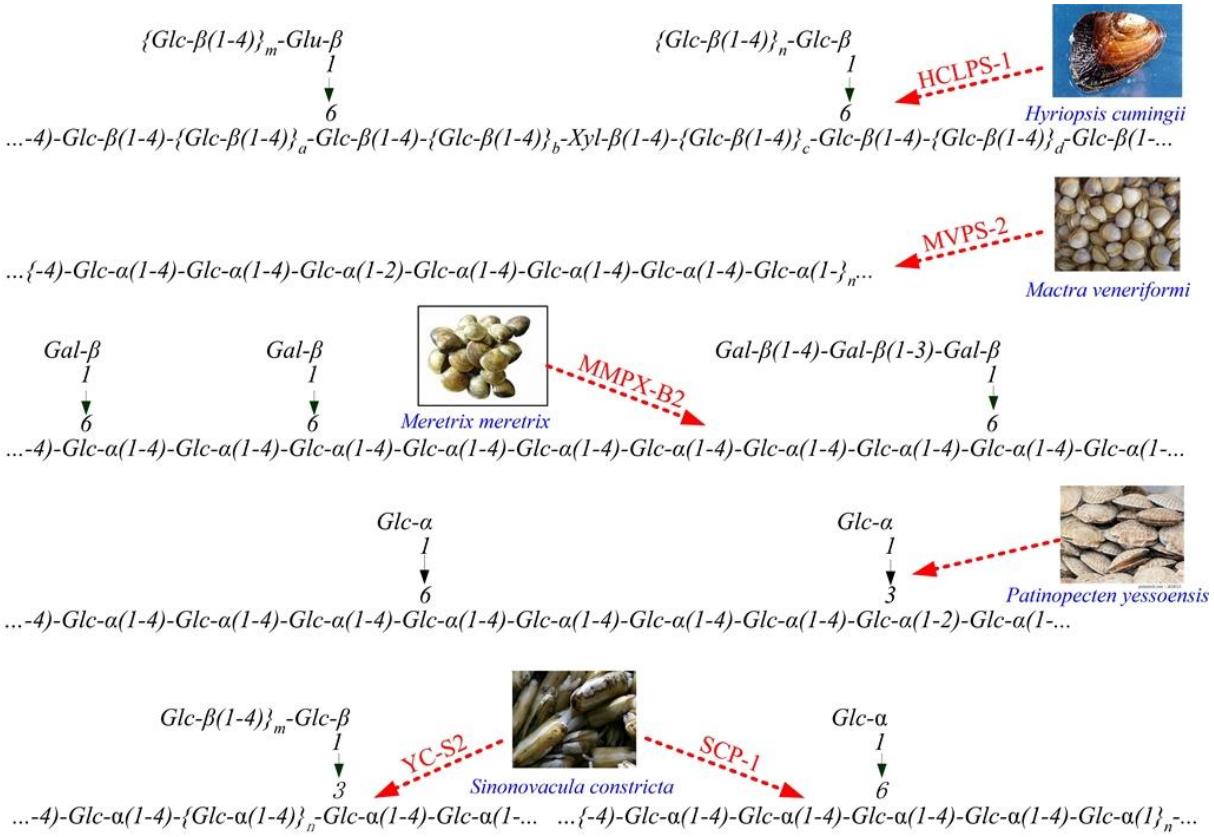

Fig. 3. The structural drawings of six glucosan compounds purified from the five representative bivalves.

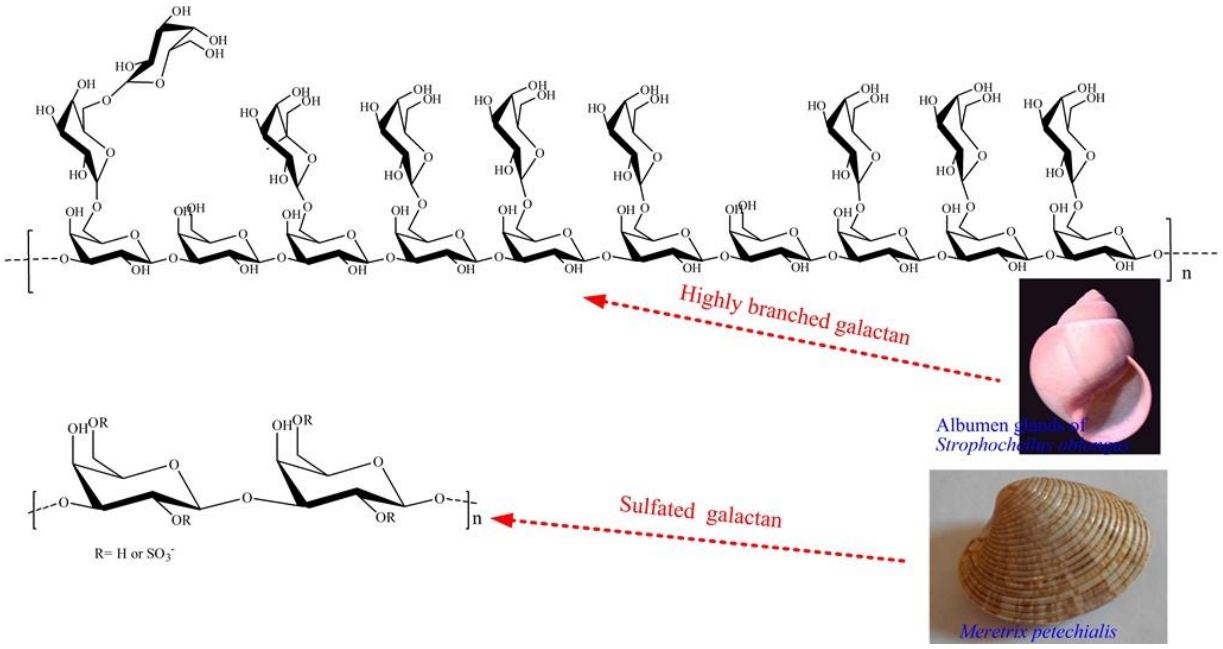

Fig. 4. The structural drawings of two galactan compounds purified from two Mollusca species.