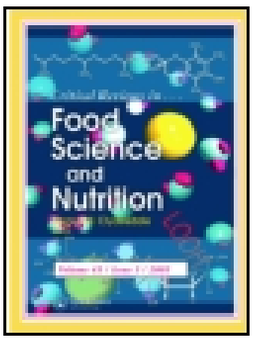

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REVIEW

Methods of extraction, separation, purification, structural characterization for polysaccharides from aquatic animals and their major pharmacological activities

Qingping Xiong[a,b,d*], Zhuoyue Song[d*], Weihui Hu[e*], Jian Liang[d], Yi Jing[b], Lian He[f], Song Huang[d], Xiaoli Wang[b], Shaozhen Hou[d], Tingting Xu[a], Jing Chen[a], Danyan Zhang[d], Yingying Shi[a], Hailun Li[c], and Shijie Li[d]

[a]Key Laboratory for Palygorskite Science and Applied Technology of Jiangsu Province, Huaiyin Institute of Technology, Huai'an, Jiangsu, PR China; [b]Jiangsu Key Laboratory of Regional Resource Exploitation and Medicinal Research, Huaiyin Institute of Technology, Huai'an, Jiangsu, PR China; [c]Nephrological Department, Affiliated Huai'an Hospital of Xuzhou Medical University, Huai'an, Jiangsu, PR China; [d]Mathematical Engineering Academy of Chinese Medicine, and School of Pharmaceutical Science, Guangzhou University of Chinese Medicine, Guangzhou, Guangdong, PR China; [e]Division of Life Science, Center for Chinese Medicine, State Key Laboratory of Molecular Neuroscience, The Hong Kong University of Science and Technology, Hong Kong, PR China; [f]School of Nursing, Guangdong Food and Drug Vocational College, Guangzhou, Guangdong, PR China

ABSTRACT

The further development of fishery resources is a hotspot in the development of the fishery industry. However, how to develop aquatic animal resources deeply is a key point to be solved in the fishery industry. Over the past decades, numerous aquatic animals have gained great attention in the development and utilization of their bioactive molecules which are of therapeutic applications as nutraceuticals and pharmaceuticals. Recent research revealed that aquatic animals are composed of many vital moieties, such as polysaccharides and proteins, which provide health benefits beyond basic nutrition. In particular, aquatic animal polysaccharides are gaining worldwide popularity owing to their high content, ease of extraction, specific structure, few side effects, prominent therapeutic potential and incorporation in functional foods and dietary supplements. Thus, tremendous research on the isolation, identification and bioactivities of polysaccharides has been carried out. This review presents comprehensive viewpoints on extraction, separation, purification, structural characterization and bioactivity of various polysaccharides from aquatic animals, such as sea cucumber, abalone, oyster and mussels. In addition, this review profiled a brief knowledge on both current challenges and future scope in aquatic animal polysaccharides field. The review will be a direction of deep processing in fishery resources, which is a hotspot, but technical bottleneck. Furthermore, the review could be served as a useful reference material for further investigation, production and application of polysaccharides from aquatic animals in functional foods and therapeutic agents.

KEYWORDS

Aquatic animal polysaccharides; isolation; identification; structural characterization; protein removal; bioactivity

Introduction

The aquatic animal is an important group in animal kingdom, including mussels, oysters, octopus and other lesser-known subgroups. Aquatic animals are widespread in oceans, rivers and lakes with huge biomass. With the discovery of new species, the number of aquatic animals gradually increased with years. Similar to other aquatic resources, aquatic animals live in a hydration buffer system, which is under a certain hydraulic pressure, with little temperature difference, limited dissolved oxygen, finite light and rich ion species at a certain ion concentration (Rajwa-Kuligiewicz et al. 2015; Caraballo et al. 2014; Ficklin et al. 2013). Because of the special living environment, the process of synthesis and accumulation of polysaccharides in aquatic animals is different from terrestrial organism, resulting in the unique structural groups and physicochemical properties of the polysaccharides. Many aquatic animals were assigned commercially and have been artificially bred or fished for food consumption due to their high nutritional value and health benefits.

Polysaccharide, a kind of macromolecule carbohydrate, is made up of more than 10 monosaccharides by glycoside bonds. Recently, polysaccharides have been found to possess a variety of biological activities and have drawn great academic attentions in biochemical and medical areas, which show a broad prospect for development as nutraceuticals and pharmaceuticals (Wu et al. 2018; Yan et al. 2015; Du et al. 2016). More importantly, many of the natural polysaccharides are found to be effective and relatively nontoxic substances (Zong, Cao and Wang 2012). Current researches have demonstrated that aquatic animals are rich in protein, amino acid, polysaccharide, etc. (Wen et al. 2010; Jiang et al.

CONTACT Jing Chen chenjing6910@163.com Key Laboratory for Palygorskite Science and Applied Technology of Jiangsu Province, Huaiyin Institute of Technology, Huai'an 223003, Jiangsu, PR China; Shijie Li lisj666@163.com Mathematical Engineering Academy of Chinese Medicine, Guangzhou University of Chinese Medicine, Guangzhou 510006, Guangdong, PR China; Hailun Li, lihailun101@sina.com Affiliated Huai'an Hospital of Xuzhou Medical University, Huai'an 223002, Jiangsu, PR China
*These authors contributed equally to this paper.

2015; Shi et al. 2016). Among them, aquatic animal polysaccharides have been attracted more attention due to their unique structures which leading to various pharmaceutical and nutritional functions. It has been well known that sulfated polysaccharides have better water solubility, more obvious opalescent, stronger freezing ability, and higher content of sulfate and the charge density (Pomin 2010). Polysaccharides of aquatic animals have a higher proportion of sulfated polysaccharide than that of terrestrial organisms. Hundreds of researches have confirmed that aquatic animal polysaccharides have anti-inflammatory (Miller et al. 1993), antithrombosis (Suleria et al. 2017), antioxidant (Li et al. 2018) and anti-tumor (Jiang et al. 2015) bioactivites.

Furthermore, the sulfated polysaccharides prepared by aquatic animals was more safety than that of terrestrial vertebrates, which has higher degree in pollution of the virus or prion (Harrop et al. 1992; Pomin and Mourão 2008). However, compared with other aquatic resources, aquatic animal polysaccharides usually link to proteins through glycopeptide covalent bonds with unusual but regular repeat unit (Pomin 2009). Because of the high protein and low fat in body tissues of aquatic animals, the binding and existence form *in vivo* and glycan structures of their polysaccharides are also different from other aquatic resources polysaccharides (algae and microorganisms, *etc.*). Therefore, the characteristics of aquatic animal polysaccharides combine both that of aquatic resources polysaccharides and non-mammal polysaccharides.

Although aquatic animal polysaccharides possess huge medicinal values, and many researches have been carried out, there is no systematic review on the present research status of their preparation, characterization and bioactivities. Thus, this review comprehensively summarized the existing extraction, separation and purification methods of aquatic animal polysaccharides (Figure 1), as well as physical and chemical characterization along with the bioactivity study of them.

Extraction of polysaccharides from aquatic animals

Studies have shown that polysaccharides exist in the skin, cartilage, secretory glands, muscles and viscera of aquatic animals (Li et al. 2016). They exist as both free polysaccharides and glycoproteins, in which polysaccharides bond to

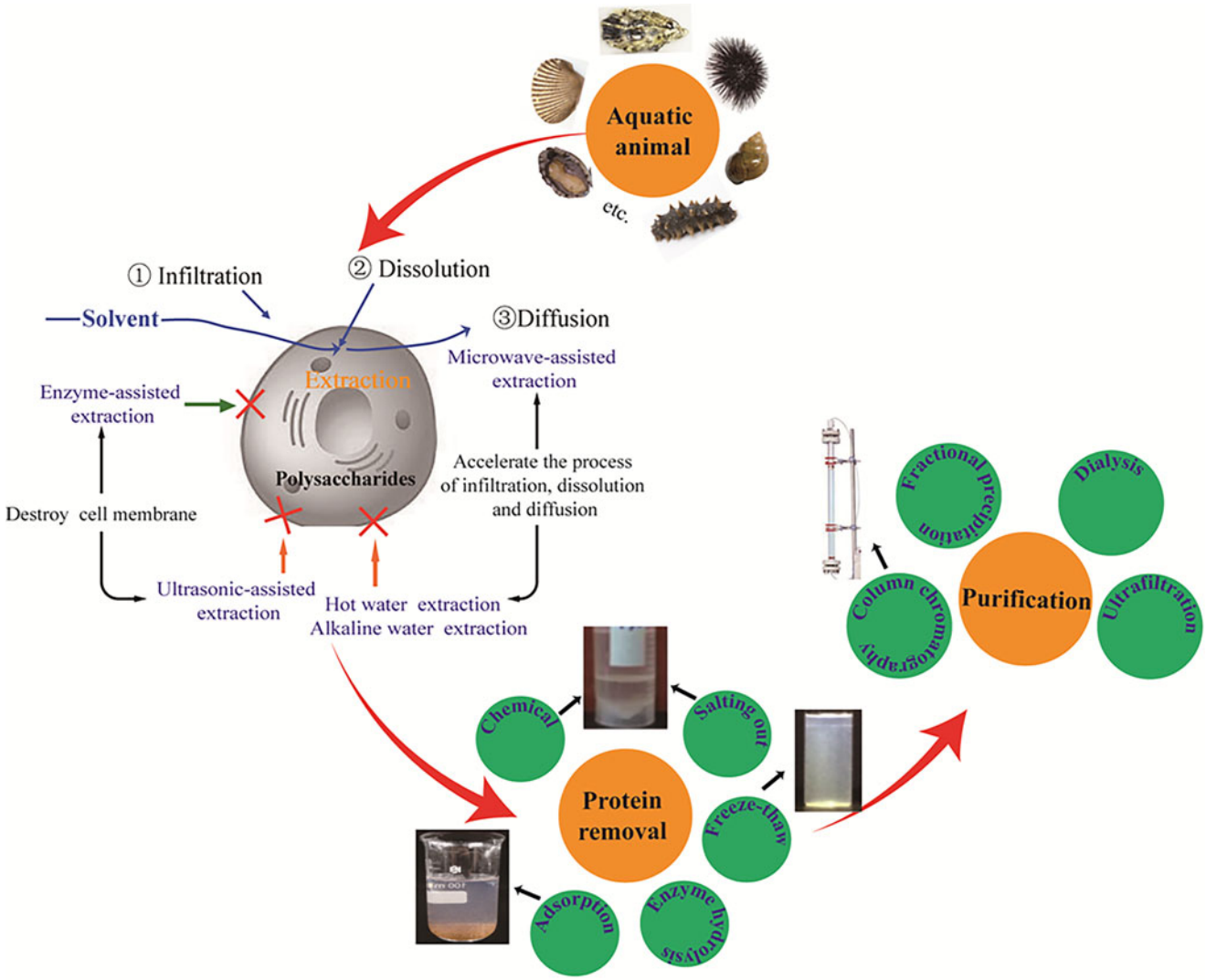


Figure 1. The schematic diagram of preparation of polysaccharides from aquatic animals (including extraction, separation and purification).

proteins by glycopeptide linkage. In addition, polysaccharides are found in cells, including cell membranes, intercellular and intracellular substance. Due to the specific properties of polysaccharides from aquatic animals, there are two main issues on their extraction process, one is how to rapidly dissociate, migrate and dissolute them, another is how to hydrolyze and dissociate them from binding proteins under the condition that polysaccharides will not significantly degrade. Therefore, different extraction methods not only exhibit different extraction speed, extraction yield and product purity, but also directly affect the species, structures and biological functions of the polysaccharides. Recently, extraction methods for aquatic animal polysaccharides with modern extraction technologies have been a research hotspot. Various extraction technologies based on different mechanisms, including hot water extraction, alkaline water extraction, ultrasonic-assisted extraction, microwave-assisted extraction and enzyme-assisted extraction are used to extract aquatic animal polysaccharides. The extraction mechanisms of different extraction methods of aquatic animal polysaccharides are shown in Figure 2. Each one has its own superiority. Selecting appropriate method is important for extraction of different aquatic animal polysaccharides

Hot water extraction

Hot water extraction is a method that could easily accelerate the diffusion rate of polysaccharides and improve the extraction efficiency with increasing temperature. The extraction process can also be further facilitated by physical methods. Firstly, an amount of purified water is added to the raw material, and then the mixture is heated to a temperature for extraction or reflux extraction. Compared with other technologies, hot water extraction process is simple, easier to control, lower cost and simple equipment. As a traditional and widely used method, hot water extraction plays an important role in the extraction of aquatic animal polysaccharides. At present, this method has been successfully used for the extraction of a variety of aquatic animal polysaccharides, such as oyster, abalone, *Stichopus japonicus*, *Cyclina sinensis*, *Glossaulax didyma*, *Cipangopaludina chinensis*, *Crassostrea gigas* (Table 1). However, hot water extraction can only extract extracellular polysaccharides, since it cannot destruct cell membrane thoroughly. In addition, although after a soaking pretreatment of the crude polysaccharides, using an endogenous enzyme could facilitate the hydrolysis of the glycopeptide bond, but the efficiency was low, thus, the production of polysaccharides was usually low.

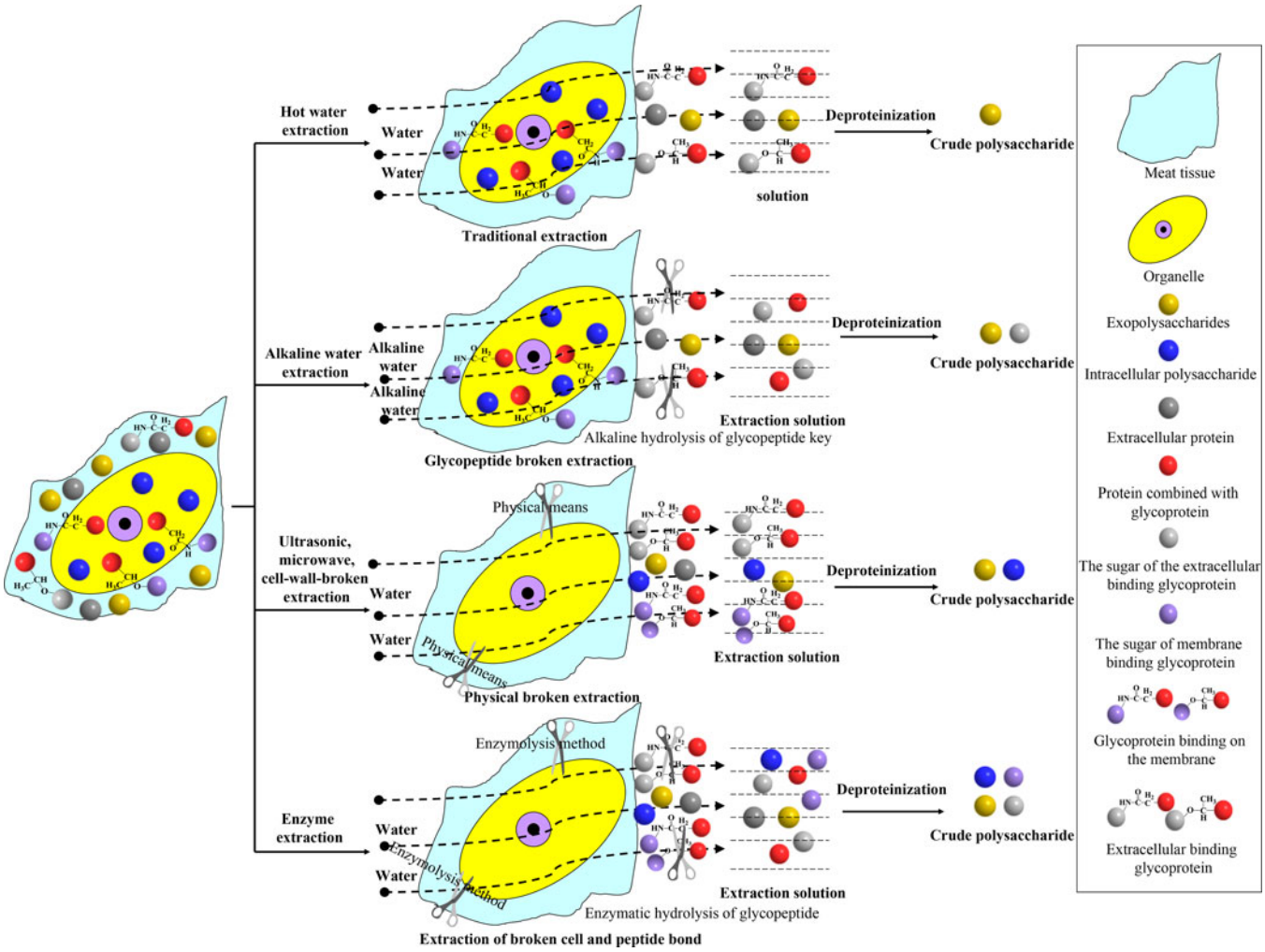


Figure 2. The schematic diagram of the extraction mechanism of polysaccharides from aquatic animals.

Table 1. Application of hot water extraction of polysaccharides from aquatic animal.

Species	Average extraction rate (%)	Literature
Stichopus japonicus	9.1	(Cao et al. 2017)
Hyriopsis cumingii	3.7	(Qiao, Hu, et al. 2009)
Mytilus coruscus	2.1	(Xu et al. 2008)
Cyclina sinensis	15.5	(Jiang et al. 2011)
Sinonovacula constricta	18.4	(Yuan et al. 2015)
Glossaulax didyma	9.1	(Li et al. 2014)
Cipangopaludina chinensis	8.7	(Jiang, Xiong, et al. 2013)
Strongylocentrotus nudus	0.56	(Liu et al. 2007)
Crassostrea gigas	2.4	(Zhong et al. 2017)
Argopectens irradias	5.4	(Tong et al. 2011)
Neverita didyma	11	(Xing et al. 2013)
Mytilus edulis	7.9	(Cheng, et al. 2010)

Alkaline water extraction

Compared with hot water extraction, alkaline water extraction can not only destroy the glycopeptide linkage of glycoprotein, but also form salt with acidic polysaccharides to increase their solubility. Thus, the yield of alkaline water extraction is much higher than that of hot water extraction and the obtained polysaccharides species are more abundant. With its simple and feasible operation, alkaline water extraction is one of the most common methods used for the extraction of polysaccharides from aquatic animals. Abalone gonad powder was dissolved in alkaline water to extract sulfated polysaccharide (Song, Zhang, et al. 2018). *Misgurnus anguillicaudatus* polysaccharides were extracted via alkaline water extraction and four different components named MAP I, MAP II, MAP III and MAP IV were successfully obtained (Zhang, et al. 2010). Polysaccharides from *Mytilus edulis* were extracted via hot water (7.9%) and alkaline water method (8.3%), indicating alkaline water method was better than hot water method (Cheng, et al. 2010). In dilute alkali solutions, polysaccharides can be released from the glycopeptide through the cracking of the glycopeptide chain (β-elimination reaction). However, high concentrations of alkaline water might destroy the glucosidic bonds, hence destroy the polysaccharides structures (Huang et al. 2009; Li et al. 2016). In addition, alkaline treatment could induce Walden conversion of the polysaccharides to the formation of 3, 6-anhydro derivatives, thus cause the desulfurization of the polysaccharides. Therefore, in order to maintain the polysaccharides structures and functional groups to the largest extent while trying to increase its yield, it is important to optimize the type of the alkaline being used as well as its concentration.

Ultrasonic-assisted extraction

Ultrasonic cavitation, mechanical forces and heat could rupture the cells and even the whole organisms. Thus, ultrasonic-assisted extraction could accelerate the release, diffusion and dissolution of effective substances in cells, and significantly improve the extraction efficiency and speed. This method directly destroyed the cells, thus, shortened the substances pathway, hence, the substances transfer was accelerated, and the extraction time was shortened. In addition, due to the broken of the cells, aside from the extracellular polysaccharides, intracellular ones could be obtained as well, exhibiting a higher polysaccharides yield. In recent years, ultrasonic-assisted extraction was widely used for the extraction of polysaccharides from aquatic animals. A natural neutral polysaccharide with antioxidant bioactivity was obtained from *Misgurnus anguillicaudatus* by ultrasonic extraction, yielding 0.02% of the fresh live loach (Qin et al. 2002a; Qin et al. 2002b). Under optimum conditions, the polysaccharide was extracted from the *Sinonov acula constricta* by ultrasound-assisted method with a yield of 8.31% (Jing 2012). However, ultrasonic-assisted extraction has no selectivity during the extraction process. Therefore, the impurity components are significantly higher than those from the hot water extraction, resulting in a more complex post purification process. In addition, high ultrasonic power can destroy glycosidic bonds and cause polysaccharide decomposition, leading to a decreased yield of polysaccharides. On the other hand, lower ultrasonic power cannot completely extract polysaccharides, thus, the extraction rate is low. The cavitation effect of ultrasonic in solution has a critical value. In the lower temperature range, the critical value of cavitation decreases with increasing temperature, and the cavitation effect is enhanced. Overall, the extraction yield of polysaccharides increases with the increasing of temperature. Over a certain temperature, the ultrasonic cavitation energy and the gas phase medium in the cavitation bubble are more easily to lose, resulting in the cavitation effect weakening or even disappearing, leading to the loss of polysaccharide extraction yield (Fu et al. 2010; Liu et al. 2006). So it is necessary to optimize the ultrasound power and time.

Microwave-assisted extraction

Microwave-assisted extraction is a new extraction method with great potential, which combines microwave with traditional solvent extraction. Substances were heated directly by microwave energy and electromagnetic energy, so that the cell membranes could be rapidly broken down, and the polarization of the molecules could be accelerated as well. As a result, the extraction efficiency could be improved with shorter time consumption. Similarly, it has been found that microwave also has a degradation effect on polysaccharide molecules (Chen, Gu, et al. 2010). Therefore, the microwave power and time should be strictly controlled during the extraction of polysaccharides. Microwave-assisted extraction had been applied in aquatic plant, such as brown seaweed (Rodriguez-Jasso et al. 2011), but seldom in aquatic animals.

Enzyme-assisted extraction

Enzyme-assisted extraction is based on the specificity of enzyme reactions, which could hydrolyze or degrade the components of cell membrane efficiently. Thus, cells could be effectively breakdown, and thereby the extraction rate of the polysaccharides could be improved dramatically, and the solvent consumption during the extraction process could be reduced to a large extent. Proteases, such as pepsin, trypsin,

papain and streptomyces, are usually selected for the extraction of aquatic animal polysaccharides, due to their eurytopicity in peptide bond breaking. This method has attracted great attention because of its stronger reaction specificity, mild operation conditions, short extraction time, high extraction rate, as well as a green and energy saving process. At present, it has been an important method for the development of traditional Chinese medicine, and has shown great application prospects. The enzyme-assisted extraction of aquatic animal polysaccharides could break the cells and selectively hydrolyze glycopeptide linkage in the premise of maintaining glycosidic bonds. Thus, the product yields could be greatly improved due to the releasing of polysaccharides from the glycoproteins. Therefore, enzyme-assisted extraction has drawn great attention from the scholars and has been the most commonly used for the extraction of polysaccharide from aquatic animals. At present, aside from the single enzyme-assisted extraction, multiple enzymes-assisted extractions, which possess combinational enzymatic effects, can provide more significantly enhanced extraction efficiency.

Vieira used papain assisted with cysteine and EDTA to extract polysaccharides from sea cucumber (Vieira and Mourão 1988). The crude *Ostrea rivularis* polysaccharides were obtained by enzyme-assisted extraction (Li et al. 2015). Pacific abalone viscera were hydrolyzed with five commercially available proteases, including alkali protease, papain, neutral protease, pepsin, and trypsin (Zhou et al. 2012). The yields of polysaccharides using papain assisted extraction isolated from four sea cucumbers, *Pearsonothuria graeffei*, *Holothuria vagabunda*, *Stichopus tremulus* and *Isostichopus badionotus*, were 11.0%, 6.3%, 7.0%, and 9.9% by weight, respectively (Chen et al. 2011). Enzyme-assisted hydrolysates from abalone were prepared using 5.0% w/w food grade papain and bromelai, with a polysaccharides content of 1.9 ± 0.7 mg/g (Har et al. 2017). The extraction of *Bullacta exarata* by papain obtained an extraction yield of 7.3% (Liu, Liao, et al. 2013). Despite of the above advantages of enzyme-assisted extraction, there are certain shortages, such as high price, strict temperature control in the process and ease to lost activity, need to be solved in future studies.

Joint extraction

Based on different mechanisms, each extraction method has its own advantages and disadvantages in extracting polysaccharides from aquatic animals. In order to obtain better extraction effects, different extraction strategies are combined, among which, enzyme extraction assisted by other methods is in dominant. The glycosaminoglycans from squid cornea were obtained by a combination method of alkaline water and enzyme-assisted extraction (Karamanos et al. 1991). The crude polysaccharide, as a water-soluble extract, was obtained from *Haliotis discus hannai* Ino viscera by alkaline protease extraction (Zhu et al. 2008). Three sulfated polysaccharides were extracted from *Bullacta exarata* using alkaline water combined with two enzymes-assisted extraction (Zhang et al. 2012). Optimized ultrasonic extraction before protease hydrolysis was a great help to improve *Crassostrea hongkongensis* polysaccharides yield and purity (Cai et al. 2014). The joint extraction method is suitable for aquatic animal polysaccharides, which are macromolecular aggregates in spatial conformation through non-covalent bonds. It shows a good development prospect and is worthy for further exploration and popularization.

In summary, the green and energy saving enzyme-assisted extraction or enzyme-assisted extraction combined with other methods were most widely applied in aquatic animals.

Separation and purification of polysaccharides from aquatic animals

Natural polysaccharides usually do not exist singly in aquatic animals, but conjugate with other components, such as proteins, lipids and nucleic acids. Compared with polysaccharides from plants and fungus, contents of pigments and small molecular impurities in aquatic animals are lower, which could be removed by repeated dissolution and precipitation. The characteristics of proteins are similar to those of water-soluble polysaccharides. Thus, proteins are the most prominent impurities in crude polysaccharide products from aquatic animals in water medium. In addition, polysaccharides are polymerized carbohydrates, their components are complex, hence their separation and purification process are different from those of small molecular compounds. In addition to the removal of non-polysaccharides impurities, the subsequent structure identification and bioactivity study require a further refining classification of the subsets of polysaccharides. Thus, it is crucial to get each individual fraction from the extracted polysaccharides with homogenous physical and chemical properties. In recent years, a great deal of research has been carried out on the removal of proteins and the subsequent polysaccharides fractions refinement. As a result, series of effective processes have been established.

Progress in the removal of proteins from aquatic animal polysaccharides

Physicochemical properties of proteins are similar to those of polysaccharides. Polysaccharides from aquatic animals always contain high content of proteins, which bring difficulty to further purification and structural analysis. In addition, most polysaccharides of aquatic animals are covalently bonded to proteins, forming macromolecule proteoglycans. Thus, the key point here is to remove proteins to the largest extent while retain polysaccharides as much as possible. The present methods for proteins removal are mainly chemical methods, enzyme hydrolysis methods as well as some new technologies. The mechanism diagrams of different deproteinization methods are presented in Figure 3.

Chemical methods

Chemical methods include traditional trichloroacetic acid (TCA) method, Sevag method and D-gluconic acid-δ-lactone method. The mechanism of TCA method involves its

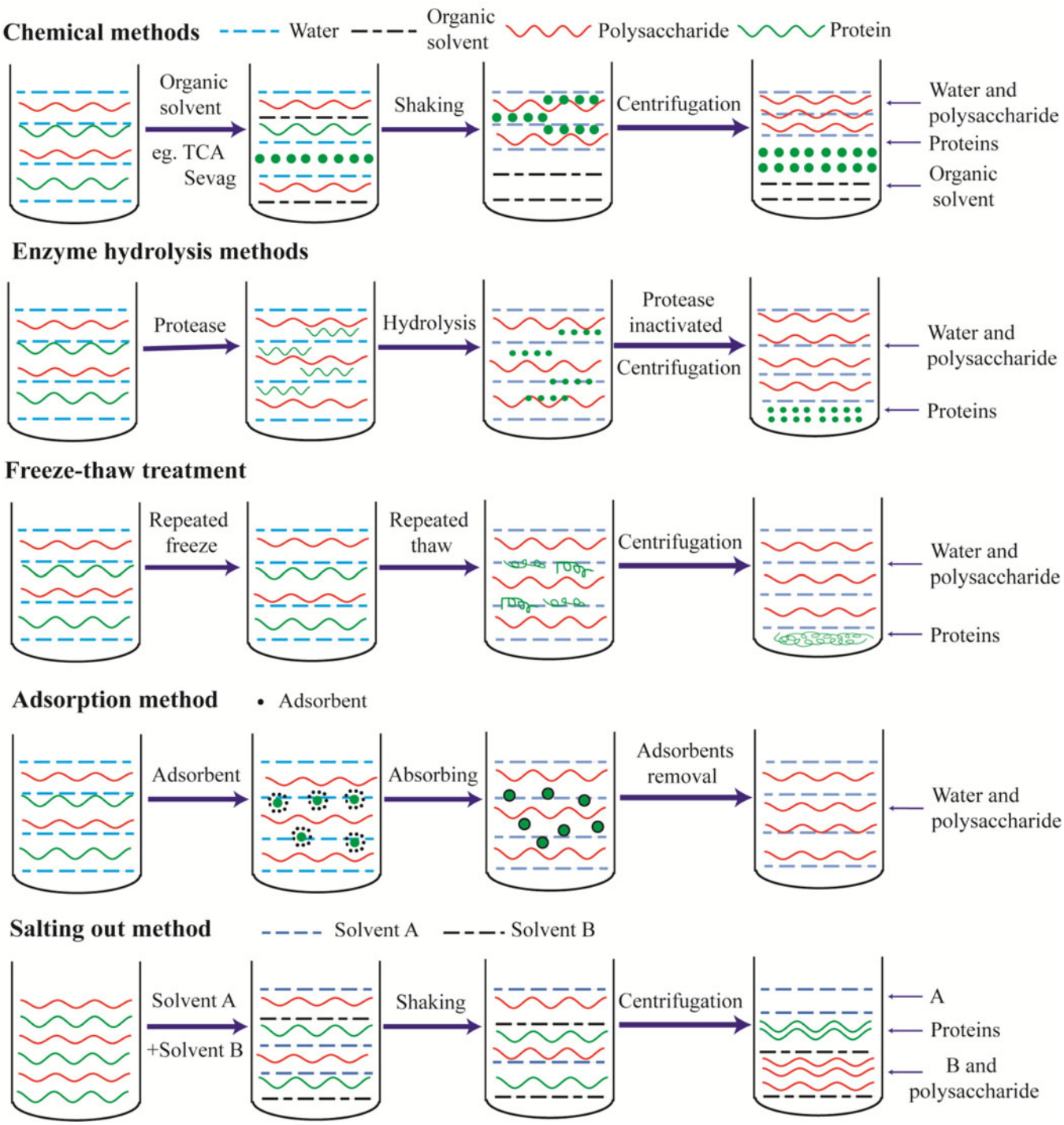


Figure 3. Mechanism diagrams of different deproteinized methods of polysaccharides from aquatic animals.

formation of insoluble salts with proteins under acidic conditions, and as a denaturant, it could induce the conformation change of proteins, resulting in the exposure of large amounts of hydrophobic groups, leading to the precipitation and aggregation of proteins. The efficiency of this method is high and it is applied in the removal of proteins from polysaccharides of mussel (Maccari et al. 2004) and *Stichopus japonicus* (Kariya et al. 1990). The Sevag method is the most commonly used and classical method in proteins removal (Sevag et al. 1938). Based on the characteristics of proteins which could denature in chloroform and other organic solvents, crude polysaccharides were mixed with Sevag reagent (chloroform: n-butanol = 5:1) and jolted. An emulsion layer, formed in between the Sevag reagent layer and extract layer, is the proteins denaturant. Thus, proteins could be removed by a separation funnel. The advantage of the Sevag method is its mild operation condition, which makes it be widely used in the removal of proteins from aquatic animal polysaccharides, such as *Cyclina sinensis* (Jiang et al. 2011), *Monetaria moneta* (Yuan et al. 2018), *Arca subcrenata Lischke* (He et al. 2007) and *Stichopus japonicus* (Cao et al. 2017). Incidentally, the method based on protein coagulant D-gluconic acid-δ-lactone in aquatic animal proteins removal is seldom.

Although these chemical deproteinization methods could accomplish high protein-removing rates and play an important role in the development of obtaining aquatic animal polysaccharides, the shortages of them are not ignorable. For instance, TCA method may cause the degradation of polysaccharides due to the severe deproteinization procedure. D-gluconic acid-δ-lactone, which composed only of carbon, hydrogen and oxygen, may bring influences on later analysis of polysaccharides. Besides, the Sevag method does not show good removal efficiency, thus requires repeated operations, hence causing much loss of polysaccharides and large amount of toxic chemical reagents residuals.

Enzyme hydrolysis method

Enzyme hydrolysis process involves the enzymolysis of proteins into small molecular peptides, which could be removed by dialysis. Papain was used in *Sinonovacula constricta* (Yuan et al. 2015) and *Tapes phylippinarum* (Cesaretti et al. 2004) to remove proteins. However, the efficiency of enzyme hydrolysis is low, so it is better to combine with chemical deproteinization methods. Protease-assisted Sevag method was used by Zhang (Zhang et al. 2012) for the removal of proteins, resulting a high protein removal rate as well as a good polysaccharides retention rate. The polysaccharide from *Strongylocentrotus nudus* eggs was deproteinated by a combination method of papain enzymolysis and Sevag (Liu et al. 2007). Crude polysaccharides obtained from *Haliotis Discus* using protease-hydrolysis and boiling-methanol extraction method contained 6.1% of proteins, while the value was 18.6% by protease-hydrolysis, water extraction and ethanol precipitation (Zhu et al. 2010; Zhu et al. 2008). The combination of the two methods not only remains the high efficiency of the chemical methods, but also makes advantage of the selectivity of enzyme hydrolysis.

New deproteinization methods

With the combination of technologies in separation and purification processes, new deproteinization methods in aquatic animal polysaccharides have emerged, such as freeze-thaw treatment, adsorption, three-phase partitioning and salting out. Freeze-thaw treatment (Xiong et al. 2017) is a green method for deproteinization of polysaccharides. Comparing with the Sevag method, it is non-pollutional, and has a higher polysaccharides recovery ratio as well as a comparable deproteinization ratio Three-phase partitioning is a simple, efficient, and green bioseparation technique based on salting out mechanism. It was used for efficient extraction and separation of polysaccharides from *Corbicula fluminea* (Yan et al. 2017). The adsorption method, is green, environmental benign and pollution-free (Song, Hu, et al. 2018), which should be further studied. These new deproteinization methods have unique advantages in protein removal rate, polysaccharide retention rate and safe operation process. Thus, it is necessary to do more research in these methods, which shows great prospect in the extraction and separation of aquatic animal polysaccharides.

Progress on purification of polysaccharides from aquatic animal

The purity of polysaccharides could be improved by removing proteins and other impurities. However, polysaccharides are composed of different subsets of polysaccharides with different physicochemical properties, monosaccharide compositions, linkage structures and molecular weights. Therefore, it is necessary to further separate these components to analyze their structures and evaluate their biological activities. The separation methods of aquatic animal polysaccharides which so far have been applied are fractional precipitation method, dialysis method, ultrafiltration method and column chromatography method. The principles of the separation methods are summarized in Figure 4A and B, and the representative examples and effects are shown in Table 2.

However, due to the different mechanisms of fractionation and purification, the properties and functions of each subclass polysaccharides fractions obtained are significantly different. The homogeneity of the same subclass polysaccharides refers only to the uniformity of one aspect of the properties that determined by the principle of the fractionation method, not all the properties. For example, the solubility of the same subclass polysaccharides obtained by ethanol fractional precipitation method is similar, but their molecular weight distribution and monosaccharide compositions are not homogeneous. We could obtain subclasses polysaccharides with similar properties, structures or functions based on different separation and purification principles. There are no advantages or disadvantages between each method, they are all reasonable. Therefore,

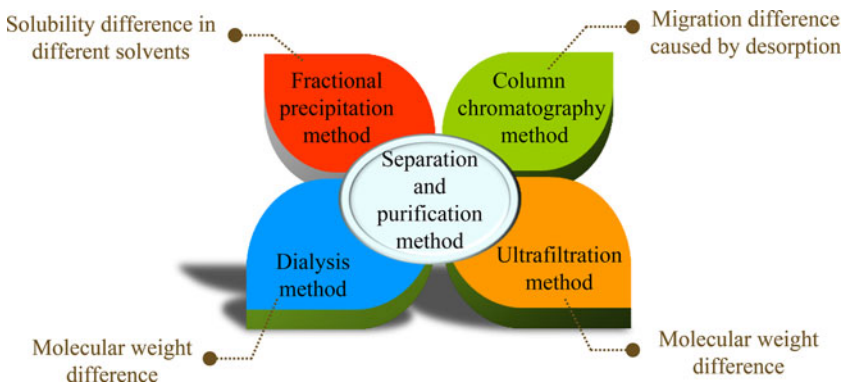


Figure 4. The principle (A) and mechanism diagrams (B) of the separation and purification methods of polysaccharides from aquatic animals.

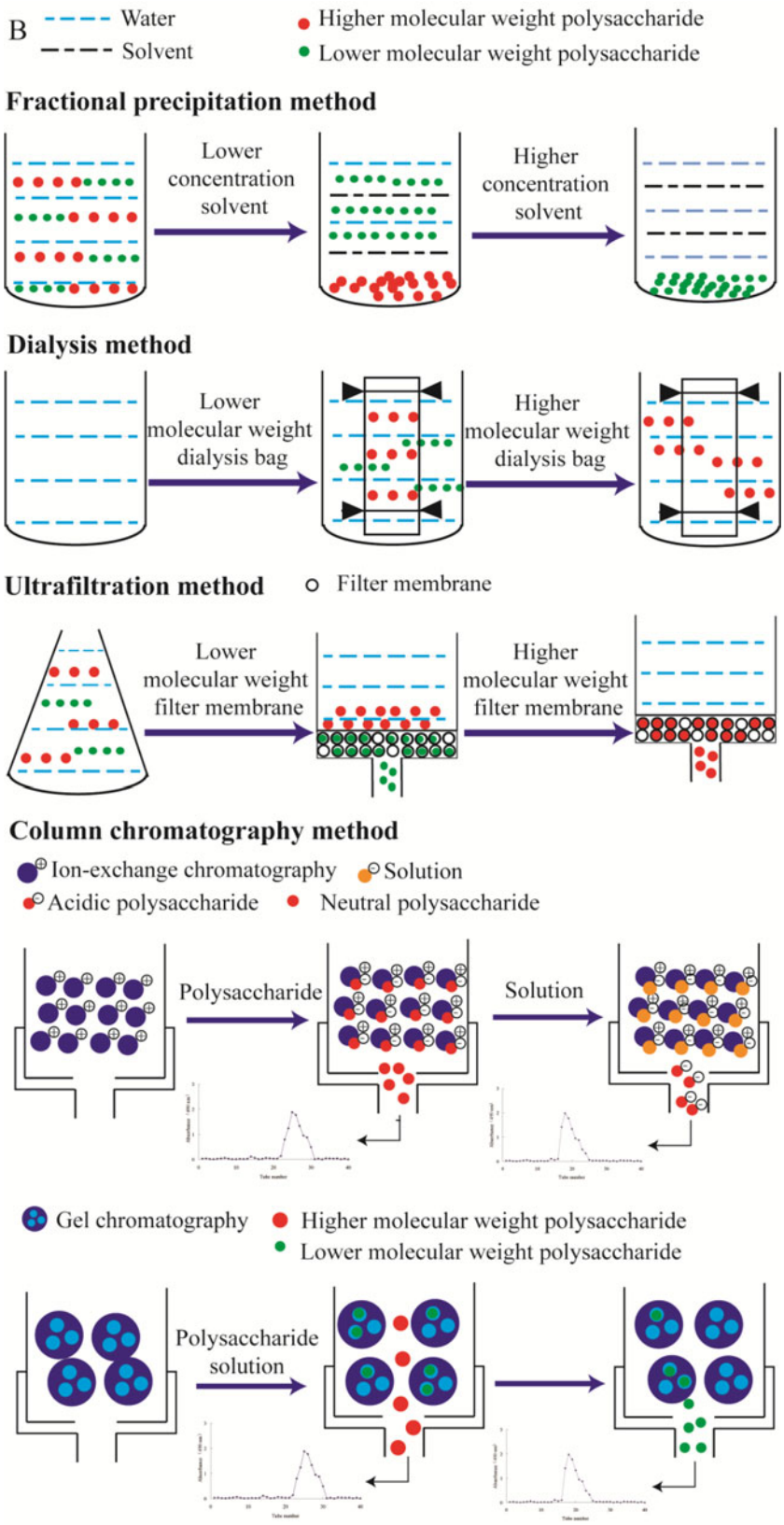


Figure 4. Continued

fractionation and purification method should be selected according to research purposes and further applications, rather than more modern, simpler or faster. For the further structural analysis and functional research, it is better to fractionalize the polysaccharides to more subtle levels that each fraction would be more uniform in their properties and functionalities. Thus, the combination of different fractionation methods is developed.

The ion-exchange chromatography (e.g. DEAE-52 cellulose) and gel chromatography (e.g. Sephdex G-100) have been

Table 2. The representative example and effect of separation and purification methods.

Separation and purification methods		Representative examples	Effect of separation and purification
Fractional precipitation method		*Apostichopus japonicus* (Liu et al. 2012)	One fraction: AJP
		Crassostrea hongkongensis (Cai et al. 2016)	Three subfractions: $C_{0-30\%}$, $C_{30-60\%}$ and $C_{60-90\%}$
Dialysis method		*Styela plicata* (Albano and Mourão, 1986)	Three fractions.
		Crenomytilus grayanus (Ovodova et al. 1992)	Contained 5% of protein.
		Corbicula fluminea (Yan et al. 2017)	The purity increased from 13.83% to 86.51%.
Ultrafiltration method		*Crassostrea gigas* (Shi et al. 2015)	A water-soluble polysaccharide: CGPS-1
Column chromatography method	Ion-exchange chromatography	*Monetaria moneta* (Yuan et al. 2018)	Three fractions: MM-P 1, MM-P2, MM-P3.
		Mussel (Maccari et al. 2004)	DP 4 to approx. DP 30
		Metriatyla scabra glycosaminoglycans (Liu et al. 2002)	Two fractions: P-1, P-2
	Gel chromatography	*Cyclina sinensis* (Jiang et al. 2011)	Three fractions: CSPS-1, CSPS-2, CSPS-3
	Ion-exchange combined gel chromatography	*Glossaulax didyma* (Li et al. 2014)	Three fractions: GDPS-1, GDPS-2, GDPS-3
		Hyriopsis cumingii (Qiao, Hu, et al. 2009a)	Three fractions: HCPS-1/2/3
		Abalone (Zhao et al. 2016)	Three sub-fractions
		Cipangopaludina chinensis (Xiong et al. 2013)	Two purified fractions CCPS-1 and CCPS-2

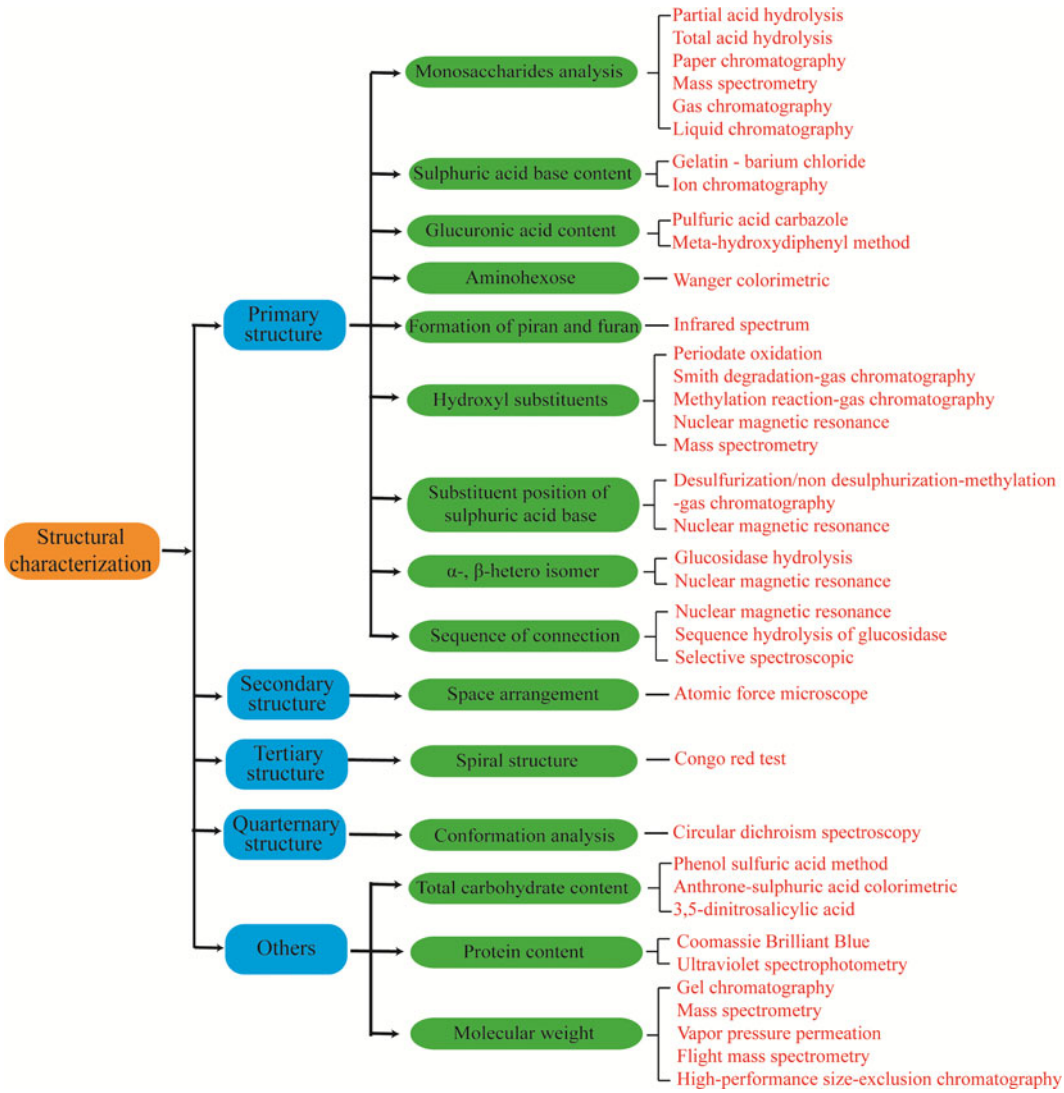
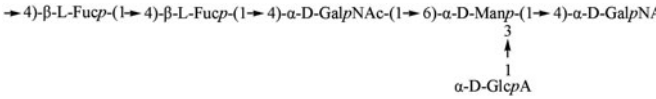
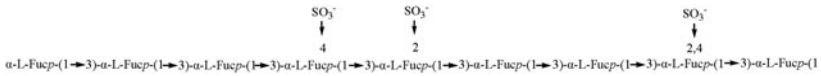
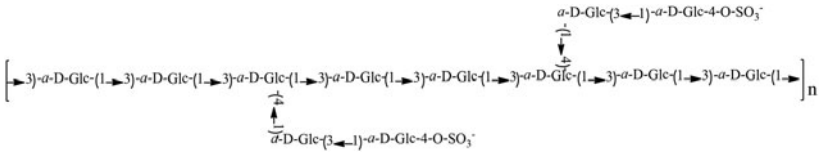
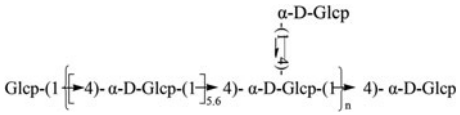
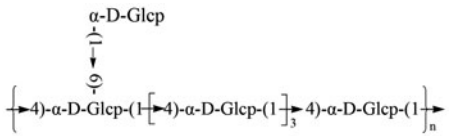
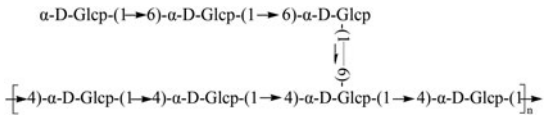
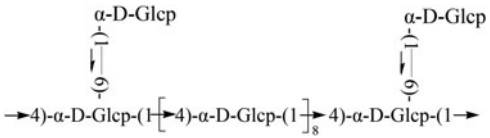
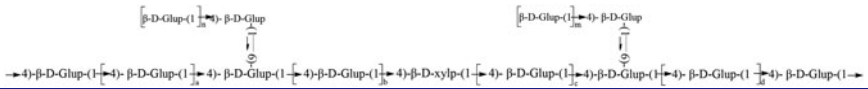


Figure 5. Common methods for structural information of polysaccharides from aquatic animals.

Table 3. Structural characterization of polysaccharides from aquatic animal.

Species	Structure	Structural information	Literature
Sepiella maindroni		Molecular weight: 11.3 KDa; Ratio of composition: glucuronic acid, mannose, N-acetylgalactosamine, and fucose (1:1:2:2)	(Liu et al. 2008a)
Stichopus japonicus		Ratio of composition: fucose (94.8%), mannose (0.3%), glucose (0.6%) and galactose (4.3%)	(Cao et al. 2017)
Cipangopaludina chinensis		Molecular weight: 91.1 kDa Composition: d-glucose (d-Glc)	(Shi et al. 2016)
Ostrea talienwhanensis		Molecular weight: 58 kDa Composition: glucose	(Yang et al. 2013)
Sinonovacula constricta		Molecular weight: 15.63 kDaComposition: glucose	(Yuan et al. 2015)
Arca subcrenata Lischke		Molecular weight: 3.5 kDa Composition: glucose	(He et al. 2007)
Strongylocentrotus nudus		Molecular weight: 1950 kDa Composition: glucose	(Liu et al. 2007)
Hyriopsis cumingii Lea		Molecular weight: 156 kDa Ratio of composition: glucose and xylose (35:1)	(Dai et al. 2009)

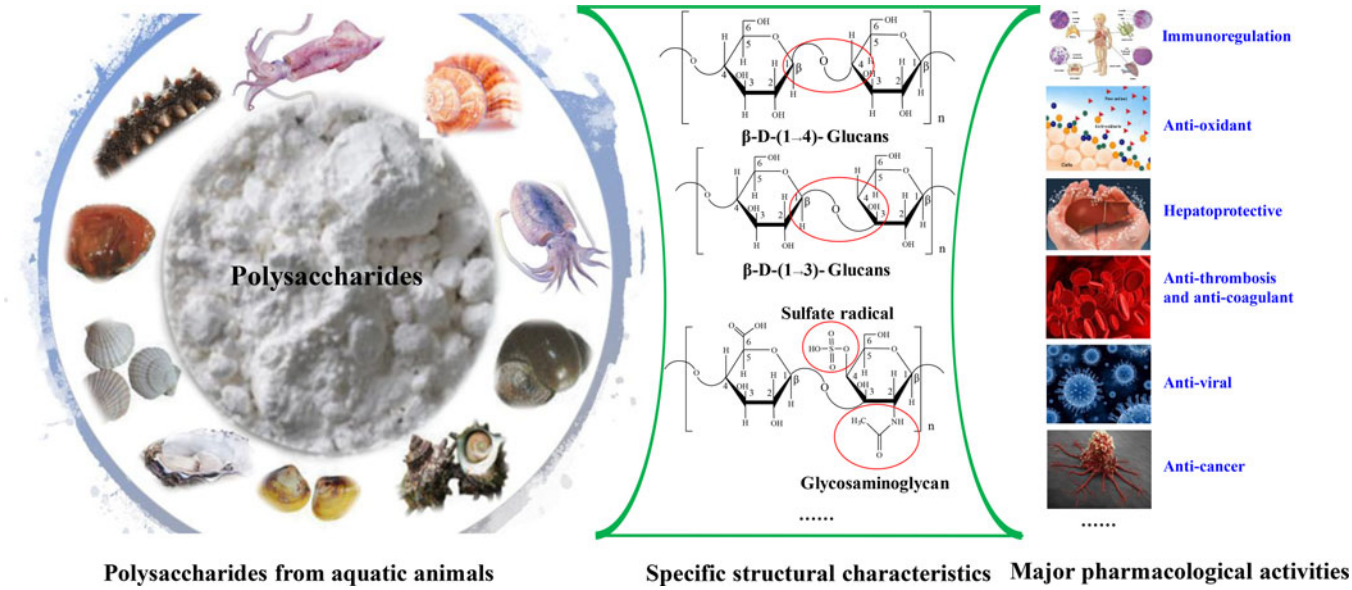


Figure 6. Specific structural characteristics and major pharmacological activities of polysaccharides from aquatic animals.

Table 4. Pharmacological activity of polysaccharides from aquatic animals.

Pharmacological activity	Polysaccharides from aquatic animals
Immunoregulation	oyster polysaccharides (Zhong et al. 2017; Achour et al. 1997; Cai et al. 2016), clam *Mercenaria campechiensis* polysaccharides (Schmeer and Beery, 1965), *Strongylocentrotus nudus* eggs polysaccharides (Liu, Xi, et al. 2008), *Hyriopsis cumingii* polysaccharide (Dai et al. 2009; Qiao, Luo, et al. 2010), Abalone polysaccharides (Zoysa et al. 2009), *Misgurnus anguillicaudatus* polysaccharide (Zhang and Huang 2005a), *Cipangopaludina chinensis* polysaccharide (Xiong et al. 2013), *Glossaulax didyma* polysaccharides (Jiang et al. 2013c), *Stichopus japonicus* fucan (Cao et al. 2017), barramundi lipopolysaccharide (Zoccola et al. 2017), *Sinonovacula constricta* polysaccharide (Yuan et al. 2015)
Anti-oxidant	*Misgurnus anguillicaudatus* polysaccharide (Zhang et al. 2011), *Bullacta exarata* polysaccharide (Zhang et al. 2012), *Hyriopsis cumingii* polysaccharides (Qiao, Ke, et al. 2009), oyster polysaccharide (Li et al. 2018; Cai et al. 2014), *Cyclina sinensis* polysaccharides (Jiang, Zhao, et al. 2013), *Monetaria moneta* polysaccharides (Yuan et al. 2017), *Corbicula fluminea* polysaccharides (Yan et al. 2017)
Hepatoprotective	*Mytilus coruscus* polysaccharide (Xu et al. 2008), *Cyclina sinensis* polysaccharides (Jiang, Zhao, et al. 2013), *Glossaulax didyma* polysaccharides (Li et al. 2014), *Cipangopaludina chinensis* polysaccharides (Jiang, Xiong, et al. 2013), *Misgurnus anguillicaudatus* polysaccharide (Qin et al. 2002b), oyster polysaccharide (Lin et al. 2017; Watanabe et al. 2016; Shi et al. 2015)
Anti-thrombosis and anti-coagulant	abalone polysaccharide (Zhao et al. 2016; Suleria et al. 2016; Suleria et al. 2017), sea cucumbers polysaccharide (Chen et al. 2011)
Hypolipidemic	*Apostichopus japonicus* polysaccharide (Liu et al. 2012), *Metriatyla scabra* Glycosaminoglycans (Liu et al. 2002)
Anti-viral	Clam polysaccharide (Woo et al. 2001), *Crenomytilus grayanus* polysaccharide (Tsybul'Skiă et al. 1992),
Anti-cancer	*Misgurnus anguillicaudatus* polysaccharide (Zhang and Huang 2005b), *Sepiella maindroni* polysaccharide (Wang et al. 2008), *Ruditapes philippinarum* polysaccharide (Zhang et al. 2008), squid ink polysaccharide (Chen, Gu, et al. 2010; Jiang et al. 2018), Abalone polysaccharide (Wu et al. 2013), *Hyriopsis cumingii* polysaccharides (Qiu et al. 2010), *Bullacta exarata* polysaccharide (Liao et al. 2017), abalone polysaccharide (Sun et al. 2010), starfish polysaccharide (Nam et al. 2006), *Strongylocentrotus* nudus eggs polysaccharide (Wang et al. 2011; Liu et al. 2007), *Cyclina sinensis* polysaccharide (Jiang et al. 2015)
Anti-angiogenesis	Squid ink polysaccharide (Chen, Gu, et al. 2010), sea cucumber polysaccharide (Tapon-Bretaudière et al. 2002)
Hypoglycemic	Meretrix polysaccharide (Yuan and Yuan, 2007)
Anti-hypertensive	*Crassostrea gigas* polysaccharide (Wang et al. 2016)
Anti-inflammation	Pacific oyster polysaccharide (Cheng et al. 2016), *Perna canaliculus* polysaccharide (Miller et al. 1993)
Antiglycation	*Misgurnus anguillicaudatus* polysaccharide (Zhang et al. 2010)
Neurosphere formation	*Stichopus japonicus sulfated* polysaccharide (Zhang, Song, Song, et al. 2010; Zhang, Song, Liang, et al. 2010c)
Osteogenic capability	Pacific abalone polysaccharide (Song, Zhang, et al. 2018), *Stichopus japonicus* polysaccharide (Kariya et al. 2004)

combined and applied widely to obtain the subclass polysaccharides with homogeneous ionic electricity and molecular weight distribution. When the crude polysaccharide solution was eluted according to polar using an ion-exchange chromatography, the different collection components were then used a gel chromatography for secondary separation according to the molecular weight, thereby obtaining the purified polysaccharide with similar polarity and uniform molecular weight. Polysaccharides from aquatic animals mostly are acidic polysaccharide, which could elute by salt concentration gradient elution using ion-exchange chromatography.

Structural characterizations of polysaccharides from aquatic animals

Material quality assessment, biological activity assessment and structure-activity relationship analysis require the precise

structure and conformation of the material. The basic components of polysaccharides are monosaccharaides, which are linked together by the glycosidic bond forming through the dehydration of the monosaccharaides. The linkage of the glucoside bonds could be either linear or branched. Polysaccharides structures are divided into four levels, namely, primary, secondary, tertiary, and quaternary structure. The primary structure mainly refers to the composition, sequence, connection, anomeric configuration and sugar chain of monosaccharide residues, as well as the location and length of the branches. The secondary structure refers to the polymer formed by polysaccharides skeleton chains which are linked by hydrogen bonds. The tertiary structure is the ordered and spatial conformation formed through non-covalent bonds of the hydroxyl, carboxyl, amino groups of the sugar residues of polysaccharides (Peters et al. 1993). The quaternary structure, the aggregates of the polymers formed through non-covalent interactions (Petersen et al. 2014). The upper level structures are dependent on the previous level structures, thus, the primary structure is the fundamental information for the analysis of higher levels structures.

However, due to the abundant monosaccharide compositions, aplenty connection sites between sugars and the flexible and changeable conformation arrangements of the sugar chains, the structures polysaccharides are more complex than those of proteins and nucleic acids. Due to this complexity, although polysaccharides structures have been studied for a long time, the relevant research accomplishments are still less than those of proteins and nucleic acids.

Fortunately, with the development of glycobiology, new technologies were applied to the structural analysis of polysaccharides. As a result, methods such as chemical analysis method, enzymatic method and instrumental method were developed and the structural analysis of polysaccharides was progressing to a new stage (Sanghi and Dhar, 2001). Common methods for structural analysis of polysaccharides are showed in Figure 5. However, it is a systematic project in the analysis of polysaccharides structures, so it is necessary to combine scientific technical methods to accomplish the analysis of the overall structures. The primary structure of polysaccharides from different resources was successfully determined by a comprehensive application of infrared spectroscopy, gas chromatography, methylation analysis, periodate oxidation, Smith degradation, and nuclear magnetic resonance spectroscopy, such as squid ink (Liu, Li, et al. 2008), *Cristaria plicata* (Zhu et al. 2012), *Hyriopsis cumingii* (Qiao, Liu, et al. 2010), *Ruditapes philippinarum* (Zhang et al. 2008, Liu, Zhao, et al. 2013), sea cucumbers (Mourão and Bastos, 1987, Cao et al. 2017), *Cipangopaludina chinensis* (Shi et al. 2016), *Cyclina sinensis* (Jiang et al. 2015), abalone (Zhu et al. 2011) (Table 3). These studies not only confirm the feasibility of structure elucidations of the aquatic animal polysaccharides but also provide a reference for the structure analysis of other aquatic animal polysaccharides.

Major pharmacological activities of polysaccharides from aquatic animal

Polysaccharides, which are widely distributed in animals, plants, fungi and microorganism, play an important role in daily dietary for the prevention of disease. In addition, polysaccharides involve in many life phenomena, such as intercellular signal transduction, cell transformation, cell division and cell regeneration. The growth environment of aquatic animals endows the special physicochemical and structural characteristics of the polysaccharides, resulting in the unique biological functions of their polysaccharides. It has been demonstrated that aquatic animal polysaccharides exhibit significant pharmacological effects on immunostimulatory, anticancer, antiviral, antioxidant, hypolipidemic, anti-angiogenic, hypoglycemic, anti-atherosclerosis, anti-thrombosis, anti-coagulant, and hepatoprotective activities (Figure 6 and Table 4). Research results have shown that these particular bioactivities of aquatic animal polysaccharides are mostly related to their unique structure (Figure 6), including types of glycosidic bond, ways of connections, distribution of molecular weight, presence or absence of sulfuric acid group, degree of sulfuric acid group and monosaccharide compositions (Li et al. 2016). For example, the polysaccharides of better anti-viral, anti-thrombosis and anti-cancer activities often contains sulfuric acid group (Lo, Jiang, Chao and Chang 2007). Meanwhile, it was also observed that polysaccharides will have stronger anti-cancer activities if their types of glycoside bond linkage are (1→3)-β-D-glucans and/or (1→4)-β-D-glucans, and molecular weight possess greater than 100 kDa (Kralovec et al. 2007). In addition, because of their good biocompatibility to human cells and no toxic side effects, aquatic animal polysaccharides show a good prospect in pharmaceutical and nutritional utilizations and developments. In recent years, with the increasing environmental deterioration and growing living standards along with life pressures, chronic diseases, cancers, cardiovascular cerebrovascular diseases and other obstinate miscellaneous diseases are overwhelming. Thus, it is an emergency to develop new medicines for the sake of human kind health. Polysaccharides from aquatic animals may become a new research hotspot due to their fabulous bioactivities, and provide new resources for the development of new drugs and functional food.

Conclusion

Because of the special living environment, aquatic animal polysaccharides have unique physical, chemical, structures and functions. There have been unique methods built up in the fields of extraction, separation and purification, structure characterization and bioactivity identification of aquatic animal polysaccharides. The enzyme-assisted method combined with other extraction methods is a worthy mentioning method for the extraction of the polysaccharides. Proteins are the main impurities in the extraction process of polysaccharides. A new, green and efficient method needs to be further developed to remove all the protein impurities. The method combined ion-exchange chromatography and gel chromatography has been applied widely to separate polysaccharides. In order to better understand the structure and function of the polysaccharides, it is necessary to select suitable methods for classification and purification according to the purpose of subsequent research. The structures of

aquatic animal polysaccharides could be well elucidated by using modern analytical instrument technologies. In addition, although the pharmacological activities of the polysaccharides from aquatic animals have been discovered, in order to make full use of the advantages of them, it is still necessary to further examine their pharmacological mechanisms and their clinical behaviors. Even so, the current widespread application and promotion of aquatic animal polysaccharides still have following several aspects limitations. Firstly, their anti-coagulation and anti-inflammatory ations have a double-sided effect. Low doses of aquatic animal polysaccharides can anti-coagulation and anti-inflammatory, but higher doses may cause bleeding and inflammation. Furthermore, because of mostly containing plenty of plasma group, such as sulfate group, the products cannot be successfully prepared due to excessive protonation when these aquatic animal polysaccharides are used as a substrate in medicines and cosmetics. The authors previous tried to add sulfated polysaccharide of *Cipangopaludina chinensis* into the cream, resulting in severe matrix collapse and stratification, which should be overcome in further applications. In addition, the current application of aquatic animal polysaccharides mostly stays in the laboratory stage, and industrial applications still need to be further expanded. Moreover, aquatic animal itself as a food has certain economic value, the extracted polysaccharides will cause little benefit when they have no special activity or weak activity. Therefore, the development and utilization of polysaccharides from waste products of aquatic animal processing products is of great significance, which can recycle resources and reduce costs.

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