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Review article

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Anti-tumor, immunomodulatory, hepatoprotective and antioxidant activity of oysters polysaccharides

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Abstract. Oyster is commonly consumed seafood, widely cultured in many regions of the world. Oyster polysaccharides have a variety of biological activities, including antioxidant, anti-tumor, immunomodulatory, antihypertensive, antiviral, anti-diabetic, anti-genotoxic, anti-allergic and antibacterial. In this review we focus on the advancements of extraction methods, structure features and bioactivities.

Keywords: oyster, polysaccharides, structure, biomedical application

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БИОТЕХНОЛОГИИ ПРОДУКТОВ ПИТАНИЯ И БИОЛОГИЧЕСКИ АКТИВНЫХ ВЕЩЕСТВ

Обзорная статья

Противоопухолевая, иммуномодулирующая, гепатопротекторная и антиоксидантная активность полисахаридов устриц

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Аннотация. Устрица – это широко употребляемый морепродукт, культивируемый во многих регионах мира. Полисахариды устриц обладают разнообразной биологической активностью, включая антиоксидантную, противоопухолевую, иммуномодулирующую, гипотензивную, противовирусную, противодиабетическую, антигенотоксическую, противоаллергическую и антибактериальную. В этом обзоре мы сосредоточимся на достижениях методов экстракции, структурных особенностях и биоактивности.

Ключевые слова: устрица, полисахариды, структура, биомедицинское применение

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1. Introduction

Oyster is commonly consumed seafood, widely cultured in many regions of the world [1]. China is the main producing country, owning 86% global production in 2016, about 100 species of oyster have been found worldwide [2]. There are 23 species of oysters in China, including Ostrea (Crassostrea) rivularis Gould, Crassostrea plicatula Gmeline, Ostrea talienwhanensis Crosse, Crassostrea zhanjiangensis sp.nov., Ostrea circumpicta Pissbry, Ostrea(Crassostrea) gigas, Ostrea （Lopha） crista-galli L., Ostrea（Lopha）hyotis L., Ostrea（Lopha）imbricata Lamarck, Ostrea Lopha）folium L., Ostrea（Lopha）sinensis Gmelin, Ostrea（Lopha）mordax Gould, Ostrea Lopha）echinata Quoy et Gaimard, Ostrea（Lopha）cucullata Born, Ostrea（Lopha）crenulifera Sowerby, Ostrea(Pycnodonta) glomerata Gould, Ostrea(Pycnodonta) cucullina Deshayes, Ostrea(Crassostrea) procellosa Valenciennes, Ostrea(Crassostrea) nigromarginata Sowerby, Ostrea(Crassostrea) paulucciae Crosse, Ostrea(Crassostrea) pes-tigris Hanley, Crassostrea hongkongensis and Ostrea denselamellosa Lischke [3, 4]. There are three kinds of common oysters

in Europe and America, including Ostrea（Ostrea）edulis Linne, Ostrea（Gryphea）angulate Lamarck and Ostrea (Crassostrea) virginica Gmelin [4]. Many reports suggest that oyster is an excellent source of high-quality nutrition, as it contains amino acids, polysaccharides, proteins, lipids and minerals [5, 6]. It has been used in traditional Chinese medicinal drugs for centuries [7] to treat low calcium levels disease such as bone loss (osteoporosis), a certain muscle disease (latent tetany), etc. One of the most important biological active substances of oyster are polysaccharides, which make it possible for application in medical purposes.

In recent years the water-soluble polysaccharides in oyster have become the object of intense interest due to their various bioactivities. Recently a large amount of results of oyster polysaccharides bioactivities have been reported, including their inhibitory effects on influenza virus reproduction[7], hepatoprotective effect [8], immunostimulatory activity [9], antioxidant activity [10], antimicrobial activity [11], anti-tumor activity [12], antihypertensive activity [13, 14] and complement activity [15].

This review presents an overview of recent progress on extraction methods, structure features and bioactivities of oyster polysaccharides. Furthermore this review will mainly focus on mechanisms of polysaccharides bioactivities in oyster.

2. Extraction methods

Most of the methods of extraction of oyster polysaccharides has been carried out by a hot water extraction and precipitation with ethanol. Oyster glycogen Type II (Sigma) was purified by precipitation with 50 % ethanol from a 2% solution in distilled water, the process was repeated three times [16]. Oyster glycogen also was separated by organic acids or enzymatic hydrolysis and precipitation with ethanol [17, 18]. Oyster-derived polysaccharides (OPS) were precipitated with an equal volume of 95% ethanol from the total extract of oysters, and then the mixture was incubated with hot water at 80oC for 4 hours [19].

Hot alkaline method was used to extract polysaccharides from C. gigas [20]. Shi and colleagues published C. gigas polysaccharides extraction process, including a hot-water extraction, isoelectric precipitation, hydrolysis and ultrafiltration [8]. Ultrafiltration process is a cheaper and more practical method for polysaccharides extraction [21, 22]. To optimize the separating of polysaccharides by using ultrafiltration membrane from C. gigas, the results of single factor and orthogonal test showed that the flux of ultrafiltration membrane with molecular weight cut off of 10 kDa was better [23]. The optimum conditions for ultrafiltration membrane were temperature at 30oC, operating pressure 0.04 MPa and pH 7.0, the polysaccharides from C. gigas cut off by membrane has molecular weight of 1.2 × 103 kDa, and the permeate liquor has molecular weight of 4.4 kDa [23]. Gao and colleagues reported that C. gigas polysaccharides were obtained from acetone powder of C. gigas by water extraction at the room temperature, 60o C and dilute alkali extraction sequentially, the polysaccharides purified by DEAE Sepharose FF anion-exchange and Sepharcryl S-400 gel-permeation chromatography, respectively [24].

Orthogonal experiment and responding surface methodology （RSM） are continuously optimized extraction process to obtain high yields of extracted oyster polysaccharides. Tian et al used orthogonal experiment to study the optimal extraction conditions of oyster polysaccharide, the results showed the optimal extraction conditions were pH 8.0 for 4 hours at 65 oC and repeated 3 times, the ratio of solid to liquid 1:15 (mg/ml) [11]. The optimal extraction conditions of oyster polysaccharide were pH 6.0, extraction for 5 hours at 75 oC, extraction for 3 times, extracting ratio was 2.56% [18]. One-factor-at-a-time (OFAT) method and response surface methodology were optimized enzymatic extraction to obtain higher yield of polysaccharides and polysaccharides on a dry basis from C. plicatula Gmeline [25]. Response surface methodology (RSM) also were optimized enzymatic extraction to obtain higher yield of polysaccharides from C. rivularis [26].

During the preparation of C. gigas polysaccharides, drying technology is key process. Hu et al. evaluated the physico-chemical properties of C. gigas polysaccharides obtained by different drying methods: freeze-drying (FD), spray-drying (SD) or rotary evaporation-drying (RED), different dry-

ing methods may affect the surface topography and structure of C. gigas polysaccharides [27]. The C. gigas polysaccharide obtained by SD is smaller and more uniform in size than the polysaccharide obtained by FD or RED and presented oval shape and smooth surface particles [27]. Ma et al. and Choi et al. had reported that smaller polysaccharides particles could lead to the changes in molecular weight, intermolecular distance and interconnection [28, 29].

3 Structure features and chemical modification characteristics

Oyster is rich in polysaccharides, and the polysaccharides can be derived from internal organ, muscle, and shell [30, 8]. Structure of oyster polysaccharides was analyzed by high performance liquid chromatography (HPLC), nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (IR), gas chromatography-mass spectrometry (GC-MS), multi-angle laser light scattering (HPSEC-MALLS) and methylation analysis [31, 32]. Herein, we list research results of oyster polysaccharides, including their monosaccharides composition, molecular weight, linkage type and bioactivities (table).

Wang et al. prepared a polysaccharide from C. gigas visceral organ, it has SO3- group shown by the analysis of FT-IR [33]. Rani et al. reported that oyster glycogen had a beta-amylolysis limit (42%) and the average chain length was 11 glucoses, the apparent ratio of A and B chains was 1.24:1 [16]. Chen and colleagues investigated the fine structure of oyster glycogen, its average chain length, interior chain length and exterior chain length were 13, 9 and 3, respectively [34]. The ratio of A and B chains in this oyster glycogen was 0.88:1.0 and the multiple branching degree was 1.88 [4]. The molecular weight of oyster glycogen was in the range of 106~107 using gel filtration chromatography on Sepharose CL-6B and Sepharose 2B, oyster glycogen was a homogeneous polysaccharide and was composed of glucose using GC [20]. The oyster-derived polysaccharides contained β-glucans, as determined by measuring its nondigestible portion to α-glucosidase, its molecular weight was approximately 435 kDa [19].

Gao et al. separated five polysaccharides from C. gigas, and the structure of MC-11 was characterized as →4)-α-D-Glc-(1→, with few →3, 4)-β-d-Glc-(1 → and →2,4)-β-D-Glc-(1→ branched units [24]. The primary water-soluble C. gigas polysaccharides was the α-configuration of D-glucan and had an average molecular weight 6.5 × 106 Da [8]. The molecular structure of polysaccharides obtained by various extraction methods was different. Results of the investigation of the structure would provide information for research of the structure-activity relationships of oyster polysaccharides.

Oyster shells contain small amounts of organic matrix: soluble matrix (SM) and insoluble matrix (IM) [30]. SM contains sulfated polysaccharides, and IM contains β-chitin, these polysaccharides tend to bind to proteins in shell [30]. Traditional Chinese medicine mainly uses oyster shells.

In addition, chemical modification can be used to improve the biological activity of oyster polysaccharides. Sulfated polysaccharides have been found to exhibit different biological activities, so they are receiving more attention. Yang et al. succeeded in preparation of Ostrea talienwhanensis Crosse glycogen (OG) with sulfation at C-6 position (SOG). The SOG exhibited splenic lymphocyte proliferation activity in vitro more strongly than OG, the activity was a dose-dependent pattern [35]. Zhao et al. accomplished to prepare C. gigas polysaccharides (CGP) with sulfation at C-6 position (SCGP). The SCGP displayed higher antioxidant activities in vitro compared to CGP, the activity was a dose-dependent pattern [36]. Using UHPL-Q-TOF-MS analysis the hepatoprotective effect of SCGP, SCGP was confirmed as decreasing total cholesterol (T-CHO), low density lipoprotein cholesterol (LDL-C), total bilirubin (TBIL), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels of serum in alcoholic liver injury mice, a total of twenty-one metabolites were screened as potential biomarkers, these results suggested that SCGP altered the amino acid metabolism, oxidative stress and immune response [36]. The SCGP protection against CCl4-induced liver injury also was confirmed using in vivo experiments [37]. The SCGP inhibited growth effect of AGS, SK-OV-3 and HepG2 cells, it could effectively induce the apoptosis of AGS cells [38].

Monosaccharides composition, molecular weight, structure features and bioactivities of oyster polysaccharides
Состав моносахаридов, молекулярная масса, особенности структуры и биологическая активность полисахаридов устриц

No.	Name	Monosaccharides composition	Mw (Da)	Structure features	Bioactivities	Ref.
1	CHP2-3	D-Glc, D-Gal, L-Ara, molar ratio: 57.80:23.86:18.34	4.181×10^4	α-type glycosidic linkages	Antioxidant, reducing oxidative damage in IEC-6 cells	[10]
2	CGPS-1	D-Glc	6.5×10^6	α-type glycosidic linkages	Antioxidant, hepatoprotective, anti-aging, antidiabetic activity	[8] [27] [39] [40]
3	CGP	D-Glc	$\sim3.413\times10^6$	Unknown	Antihypertensive activity	[13]
4	MC-11	D-Glc	1.299×10^6	→4)-α-D-Glc-(1→, with few →3, 4)-β-d-Glc-(1 → and →2,4)-β-D-Glc-(1→ branched units	Unknown	[24]
5	CGOP	Have -O-SO_3^- group	Unknown	Unknown	Antioxidant, hepatoprotective effects	[33]
6	LMW-OPS	α-D-glucopyranose	1980-2630	α-type glycosidic linkages	Activation of dendritic cells, anti-tumor actovoty	[41] [42]
7	CRP-1	D-Man, D-Ribose, Glusamine, Glucuronic acid, Galacturonic acid, D-Glc, Galactosamine, D-Gal, L-Ara, molar ratio: 55:0.32:9.08:0.47:0.66:48.13:3.57:8.53:0.30	1.245×10^5	Polyaldehyde pyranoside	Immunoregulatory activity	[43]
8	CRP-2	D-Man, D-Ribose, Glusamine, D-Glc, Galactosamine, D-Gal, molar ratio: 7.79:0.41:12.69:32.08:4.98:15.92	6.7×10^4	Unsaturated polyhydroxyketone	Immunoregulatory activity	[43]
9	CRP-3	Glusamine, D-Glc, D-Gal , molar ratio: 8.91:5.71:10.15	8.3×10^3	Polyunsaturated polyhydroxyketone with carboxyl groups	Immunoregulatory activity	[43]
10	CGPs	D-Glc	1.571×10^5	α-(1→4) configuration	Antioxidant, antihypertensive, hypoglycemic activity	[44]

4. Bioactivities

4.1 Antioxidant, hepatoprotective and anti-aging activities

Shellfish polysaccharides usually showed antioxidant activities. Many diseases were related to the free radical oxidation, such as cancer, inflammation and cardiovascular disease [45]. Therefore, shellfish polysaccharides with antioxidant is an emerging trend recently.

Oyster polysaccharides extracted by various methods have different structures and ~~hence~~ have different biological activities also or hence or too. The extraction methods affect to the structures of oyster polysaccharides and their biological activity. The antioxidant activity of oyster polysaccharides obtained by freeze-drying, spray-drying and rotary evaporation-drying, named as FDCGP, SDCGP and REDCGP, were measured and the results showed that SDCGP exhibited stronger antioxidant activities on 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) free-radical scavenging and 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging [27]. Cai et al. isolated the $C_{30\text{-}60\%}$ fraction of oyster polysaccharides (CHP2) into four fractions, namely, CHP2-1, CHP2-2, CHP2-3 and CHP2-4. These fractions are positively correlated with ABTS free-radical scavenging rate and oxygen radical absorbance capacity (ORAC), and the CHP2-3 fraction demonstrates the best antioxidant activity *in vitro* [10]. In addition, CHP2 and CHP2-3 protect IEC-6 cells from H_2O_2 damage, the protective effect of CHP2-3 on the H_2O_2-treated IEC-6 cells was related to inhibition of interleukin-1β (IL-1β) and interleukin-6 （IL-6）expression, inhibiting the attenuated nuclear factor-κB (NF-κB) signaling pathway [10]. A water-soluble oyster polysaccharide (CGPS-1) improved the qualities/amounts of ALT, AST, MDA and SOD in CCl_4/ethanol-induced liver injured mice [8]. Wang et al. extracted CGOP and confirmed that CGOP had DPPH, ABTS and hydroxyl radical scavenging activity [33]. CGOP improved the qualities/amounts of ALT, AST, malondialdehyde (MDA) and superoxide dismutase (SOD) in CCl_4-induced liver injured mice [33]. These results suggest that CGOP could effectively prevent liver injury by antioxidantive effects [33].

Aging is an irreversible phenomenon, during the process of aging, the structure and function of the tissues and organs degenerate with the increasing of age, and cause their eventual deaths [46, 47]. Scientific research have found that oxidative stress from reactive oxygen species plays a crucial role in the process of aging [48]. Numerous studies have shown that polysaccharides with antioxidant activities have anti-aging activities also [49-57]. Wang et al. analyzed the serum in mice of anti-aging activity of CGPS-1 with antioxidant activity using label-free quantitative proteomic, the results of MS data identified 19 proteins [39]. Among the 19 proteins, the up-regulated peroxiredoxin-2 (AC: Q61171) and malate dehydrogenase (AC: P14152), along with down-regulated xanthine dehydrogenase/oxidase (AC: Q00519) could improve the ability to scavenge free radicals and decrease NO. This results demonstrate that CGPS-1 prevented aging through scavenging free-radicals [39].

Strenuous exercise is a physiological stress response that can lead to an increase in free radicals of the serum and liver, and thus excessive free radicals lead to the injury of the liver. Ye and Hua confirmed that the oyster polysaccharides inhibited the levels of ALT, AST, MDA, NO, NOS and increased the levels of glutathione peroxidase (GSH-PX), SOD, catalase (CAT), Na^+/K^+-ATP and Ca^{2+}/Mg^{2+}-ATP in the serum of exhausted exercise rats. The results showed that oyster polysaccharides has protective effect on liver after exhaustive swimming training through enhancement of antioxidant enzymes activities and reduction of NOS activity [58].

4.2 Antitumor and immunomodulatory activities

Antitumor compounds had been focused in recent research. Numerous studies have shown that polysaccharides exhibit antitumor activity both *in vitro* and *in vivo*, they act as potential antitumor agents in cancer treatment [59]. Wang et al. reported that oyster polysaccharides (OPs) from *Ostrea rivularis* could significantly increase spleen lymphocyte activity in tumor-bearing mice. It suggests that OPs have antitumor activity.

OPs (25mg/ml) could also improve activity of plaque forming cells and NK cells [60]. Zhong et al. found that low molecular weight oyster polysaccharide (LMW-OPS) can increase the surface

expression of major histocompatibility complex class II (MHC-II), CD40 and CD86 in bone marrow-derived dendritic cells (BMDCs) and induce the secretion of tumor necrosis factor (INF)-α and IL-12 by activating dendritic cells (DCs) [41]. Activation of DCs is a method for tumor immunotherapy [61]. Following experiments showed that LMW-OPS relieved stemness-high tumor cell-mediated suppression of BMDC function, it restimulated the activity of DCs infiltrating tumor tissues, and the results demonstrated that LMW-OPS has anti-tumor activity too [42]. Chen et al. reported that oyster polysaccharides inhibited growth of CNE-1 cells and human vascular endothelial cells [12].

Three polysaccharides named CRP-1, CRP-2 and CRP-3 were fractioned from an enzymatic hydrolysate of *Crassostrea rivularis* by ultrafiltration, following by DEAE-52 anion-exchange column chromatography. These polysaccharides presented a immunodulatory activity in mouse macrophage line RAW264.7 [43]. Li et al. reported that oyster polysaccharide (O-P) significantly increased the thymus gland and spleen, reinforced the phagocytosis ability of the macrophage cells in mice infected by herpes simplex virus type I (HSV-1) [9]. Subsequently, Li et al. confirmed that the glycosaminoglycan from oysters? Increased the thymus gland and spleen index, further showed the phagocytosis ability of the macrophage cells in mice was infected by HSV-1[62]. Song et al. reported that oyster glycogen (OG) showed anti-complement activity *in vitro*, the maximal activity inhibitory rate of 77.38% at a final concentration of 5.0 mg/ml [15].

4.3 Antihypertensive and protective vascular activities

Cardiovascular diseases, including stroke, aneurysms and hypertension, have become a global and serious health issues which causes a variety of complications [63]. Currently, pharmaceutical dgugs are commonly used for the treating of hypertension, but they often have side effects. Therefore, finding of new antihypertensive compounds is of a great significance for the treatment of hypertension. Wang et al. reported that oyster polysaccharide (CGP) treatment led to the significant decrease in both systolic and diastolic pressures in the hypertension model Wistar rats [13]. Getachew et al. reported that oyster polysaccharide (CGPs) was extracted from *C. gigas* using subcritical water (SW) and the process was optimized by RSM, the CGPs exhibited antioxidant, antihypertensive and hypoglycemic activity [61]. Wang and Liu found that oyster glycosaminoglycan (O-GAG) enhanced the antioxidant capacity of human vascular endothelial cells (VECs), promote secretion of NO, and had protection effect on VECs injured by H_2O_2 [64]. Overall, the function of oyster polysaccharides in cardiovascular diseases is clear but the detailed mechanism need to be later studies.

4.4 Other activities

In addition to the above bioactivities, the oyster polysaccharides have other activities such as antiviral, anti-diabetic, anti-genotoxic, antiallergic and antibacterial.

Li et al. reported that oyster polysaccharide (OPS) could decrease the hemagglutination titer of influenza virus in Madin-Darby canine kidney (MDCK) cell culture, OPS and ribavirin drug combination could inhibit the proliferation of influenza virus in MDCK cell culture and had the addition effect [7]. Zhao et al. found that CGPS-1 has the hypoglycemic effect. A urine metabolomics approach (UPLC-Q-TOF-MS) applied to evaluate the effects of CGPS-1 in alloxan-induced diabetic mice, nineteen metabolites in urine such as L-carnitine, hippuric acid, pantothenate and ornithine were identified as biomarkers, they were mainly involved in amino acid metabolism, carbohydrate metabolism and purine metabolism [40]. Lin et al. reported that the polysaccharides from *Ostrea plicactula* Gmelin protected against cyclophosphamide (CP)-induced genotoxicity and hepatotoxicity in BALB/c mice, the beneficial effect depends on activation of nuclear factor E2 (Nrf2) – antioxidant response element (ARE) pathway and subsequent suppression of oxidative stress and genetic toxicity [65]. Oyster-derived polysaccharides (OPS) modulated the T helper Th1/Th2 immunobalance toward to the Th1-dominat direction in antigen-primed splenocytes *in vivo*, subsequently confirmed that the OPS has anti-inflammatory activities against food allergy [19]. Tian et al. reported

that oyster polysaccharides exhibited antibacterial activities against *Escheria coli, Staphylococcus aureus* and *Bacillus subtilis*, the activity against *S. aureus* was strongest[11].

5 Conclusions

In recent years, research reports on oyster polysaccharides from different oyster species have gradually increased, mainly focused on the extraction methods, structure features and biological activities. Numerous results have confirmed that oyster polysaccharides have biological activities such as antioxidant, anti-tumor, immunomodulatory, antihypertensive, antiviral, anti-diabetic, anti-genotoxic, anti-allergic and antibacterial activities. However, most studies have only reported biological activities of oyster polysaccharides *in vitro* and *in vivo*, but lacking the relationship between structure and biological activity of oyster polysaccharides and the mechanism of action unfortunately. Therefore, the structure-activity relationships and the underlying molecular mechanisms of biological activity of oyster polysaccharides need to be explored in the future, by which the underlaying mechanism will contribute to application in food, medical and industrial areas.

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