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Abundance of saccharides and scarcity of glycosaminoglycans in the soft tissue of clam, *Meretrix meretrix* (Linnaeus)

Lingchong Wang[a,b,*], Liuqing Di[a], Hao Wu[a,b,*]

[a] School of Pharmacy, Nanjing University of Chinese Medicine, Nanjing, 210023, PR China
[b] Jiangsu Key Laboratory of Research and Development in Marine Bio-resource Pharmaceutics, Nanjing University of Chinese Medicine Nanjing, 210023, PR China

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ABSTRACT

We investigated presence and distribution of glycosaminoglycans (GAGs) in *Meretrix meretrix* soft tissue by determining GAG composition in the different parts, namely, mantle edge, foot, gill, adductor muscle, and viscera. The occurrence of glycan ingredients was examined by histochemistry, whereas GAG and general polysaccharide contents in clam tissue were qualified through extraction and determination. Tissue sections stained with alcian blue or periodic acid–Schiff demonstrated the general existence of saccharides and trifling generation of GAGs in clam tissues. GAGs coexisting with glycogens appeared to be primarily produced in the mantle and foot tissues in mucus form by visualization. The GAG content of the polysaccharide extract ranged from 16.8 to 75.8 mg in 10 g of 5 dried tissue materials in comparison with total carbohydrate level in the range of 500–1760 mg, thereby indicating that GAGs were not the major components of polysaccharide extracts. GAG composition only accounted for approximately 4% of total glycan components, which consist of the determinations of amino sugar and uronic acid. The soft tissues of clam contained abundant saccharide compounds but sparse amounts of GAGs. The results will benefit the subsequent development of products made from the polysaccharide components of *M. meretrix*.

1. Introduction

Glycosaminoglycans (GAGs) are acidic, highly sulfated, complex, and linear polysaccharides, which often covalently bound to proteins and form proteoglycans (Volpi and Maccari, 2007). These GAGs are also called as acidic mucopolysaccharides, such as hyaluronan, keratan, chondroitin, dermatan, and heparin. GAGs have several fundamental biological and pharmacological properties and have been developed as important initial materials of medicines, cosmetics, and functional foods in modern chemical industry (Gama et al., 2006). GAGs were commonly produced from animal tissues via the processes of extraction, isolation, and purification. Commercial manufacture of GAGs relies on mammalian (bovine or porcine) tissues, such as skin, intestinal tract, and connective tissues. However, these mammalian tissues are not safe due to the risk of some viral infections. Therefore, finding alternative, non-mammalian GAG resources is important (Volpi and Maccari, 2007).

Sea constitutes an immense reservoir of unique natural molecules with potential biological activities. GAG ingredients are a group of compounds among the countless bioactive molecules of marine origin that occurred with elevated quantities. Various pharmacological properties ranging from anticoagulant and antithrombotic to anti-metastatic and anti-inflammatory can be attributed to these compounds. A comprehensive survey of different classes of invertebrates has shown that GAG analogues are present widely in many marine animal phyla, particularly for the taxa of mollusks (Wang et al., 2017). At present, increasing amount of attention have been paid on the exploration and utilization of marine mollusks, considering that many species exist in the sea, and huge biomass is available. Approximately 110,000 mollusk species are present and over 70% of them live in the sea. Exploration and product development on natural GAGs from marine mollusks has become a topic of considerable research interest. Some interesting results have been reported by worldwide experts on mollusk GAGs (Vidhyanandhini et al., 2014; Vijayabaskar and Somasundaram, 2012; Volpi and Maccari, 2005). The presence of heparin GAGs in *Mercenaria mercenaria* was confirmed by histological examination. Heparin GAGs are localized in the labial palp, ctenidia, siphons, pallium, intestine, and foot of the northern quahog bivalve (Ulrich and Boon, 2001). One GAG preparation was obtained from large freshwater mollusk bivalve *Anodonta anodonta*, which was composed with 38% chondroitin sulfate, 21% nonsulfated chondroitin and 41% heparin (Volpi and Maccari, 2007). Giant African snail *Achatina fulica*

* Corresponding authors at: School of Pharmacy, Nanjing University of Chinese Medicine, 138#, Xianlin Road, Nanjing, 210023, PR China.
E-mail addresses: 993wlc@njucm.edu.cn (L. Wang), Whao5795@vip.sina.cn (H. Wu).

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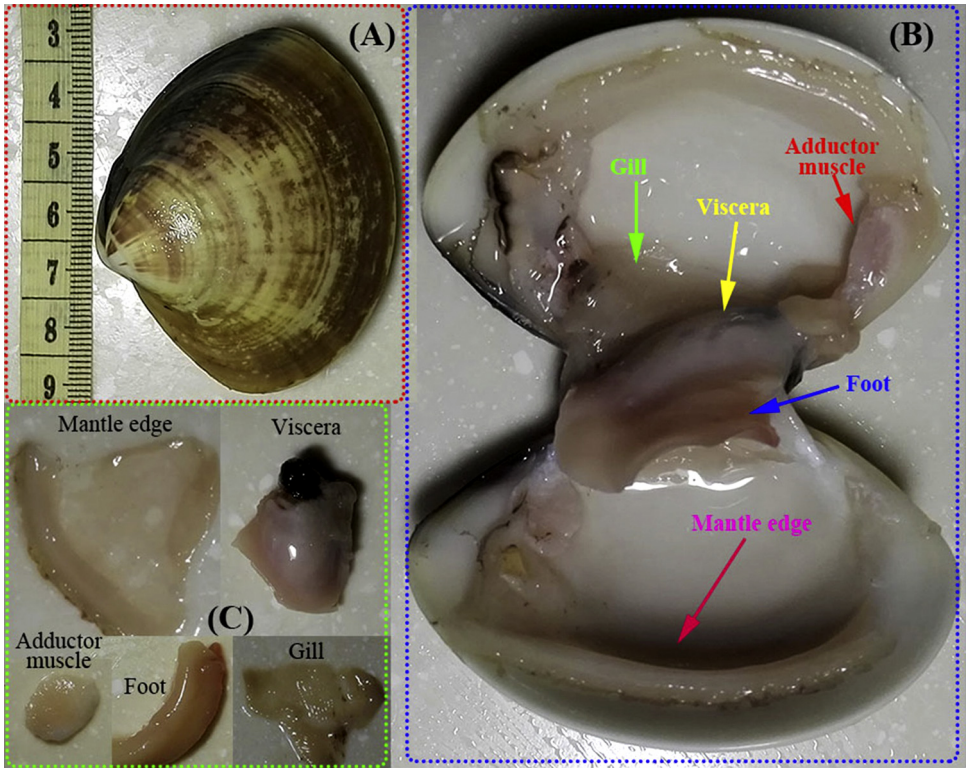

Fig. 1. External morphology (A), internal morphology (B), and five cutting tissue parts (C) of Meretrix meretrix.

contains one novel GAGs, which was named acharan sulfate and had the peculiar disaccharide sequence with ΔIdoA2S-α(1→4)-GlcNAc (Jeong et al., 2001). These achievements motivated us to study GAG ingredients generated by some mollusks, especially in specific marine species of China (Yu et al., 2008).

Meretrix meretrix (Linnaeus), which is a clam living in coastal mud flats, is an important economic species of marine bivalve and widely distributed in the shallow seas of South and Southeast Asia. This edible marine clam has now successfully achieved large-scale aquaculture in beaches along the coastline of Jiangsu, China. Approximately 200,000 ac of breading areas are available and account for 80,000 T annual yields. Except commercially exploited for food products, soft body of the clam is also traditionally used as Chinese medicine to relieve pulmonary, liver, or stomach pains. Medical records on this topic are available in some famous Chinese medicine books, such as Compendium of Materia Medica, Chinese Materia Medica, and Chinese Marine Materia Medica. Scientists who worked on marine natural products have gradually realized that the healthy benefits of this bivalve perhaps are correlated to their functional glycan compounds (Li et al., 2014). The China Food Nutrition Fact reported that carbohydrates account for approximately 10% of dried mass in body of *M. meretrix*. However, it lacks deep and systemic investigations on the glycan types and their tissue origins.

In our previous study, crude polysaccharide was extracted from soft tissue of the clam via hot water extraction combining with alcohol precipitation (Chen et al., 2016). The major component of the extract belongs to glucan analogue, but GAGs compounds are hardly detected even when it was subjected to separation and isolation. In this paper, we attempt to clarify some important issues on the amount of GAGs in soft tissues, which tissues contain GAGs, and how much GAGs are contained in *M. meretrix*. Thus, histological chemistry and several quantitative determinations were carried out within five organ tissues of the calm including mantle edge, foot, gill, viscera, and adductor. The possibility of preparing GAGs by using these organ tissues as source materials for extraction is also discussed.

2. Materials and method

2.1. Materials

The clams breaded 3 years were collected by hand picking at the Lvsi harbor of Nantong, Jiangsu province, China (32°06 N; 122°30 E) and identified as *M. meretrix* (Linnaeus) by Jiangsu Marine Fisheries Research Institute. Hematoxylin, eosin, alcian blue (AB) 8-Gx, periodic acid, and Schiff's reagent were purchased from Leica Biosystems Company (Shanghai, China). Sugar standards including D-glucose, glucosamine, glucuronic acid, and chondroitin-4-sulfate were supplied by Jiangsu Institute for Food and Drug Control (Nanjing, China) and were of the highest grade available. Blyscan™ GAG assay kits (1,9-dimethyl-methylene blue colorimetric principle) were commercial product of Biocolor Ltd. Co. (Belfast, Northern Ireland). Sulfamate, 2-hydroxydiphenyl, acetylacetone, anthrone, cysteine, and papain were purchased from Nanjing Chemical Reagent Co., Ltd (Nanjing, China). All the other chemicals were purchased from Sigma-Aldrich Co. (Shanghai, China) and were of the highest grade available. Flat-bottomed 96-well microtiter plates and plastic seal covers for microtiter plates were obtained from Sigma-Aldrich Co. (Shanghai, China). A SpectraMax M5e microplate reader (Molecular Devices, USA) with SoftMax Pro software was used, which has PathCheck Sensor technology, to normalize the absorbance automatically with respect to volume.

2.2. Preparation of tissue sections and staining examination

Clams alive were starved in an aquarium for 24 h to evacuate their gut contents before using. Shells were opened, and soft tissues were dissected into five organ parts, including mantle edge, foot, gill, adductor muscle, and viscera by their morphological differentiation. The divided organ tissues were fixed overnight in 10% phosphate-buffered neutral formalin and then embedded in paraffin wax after dehydrating through a graded ethanol series (from 80% to 100%) and n-butyl