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# Characterization of organic matrix extracted from fresh water pearls

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ABSTRACT

Aragonite pearl and vaterite pearl from cultured *Hyriopsis cumingii* in Zhuji (Zhejiang province, China) were chosen for the study. The matrix proteins were extracted using water and weak acid, and classified as water soluble matrix (WSM), acid soluble matrix (ASM) and acid insoluble matrix (AIM). The proteins from both pearls were characterized by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), X-ray diffraction (XRD) and Fourier transformation infrared spectra (FTIR). The results showed that, AIM of aragonite pearl and vaterite pearl had an ordered structure of α-helix. ASM conformations of these two pearls were different from each other. WSM differed the most between these two pearls.



## 1. Introduction

Fresh water pearls of China are mostly originated in the middle and lower reaches of Changjiang (Yangzi) River. The majority of fresh water pearls have luster. Generally, the inorganic component of these lustrous pearls is constituted by aragonite crystal and these pearls are regarded as aragonite pearls. While some pearls that have vaterite crystal as inorganic component are viewed as vaterite pearls and most of these pearls are lack of luster [1–3]. Pearl is a kind of natural bio-composite resulting from biomineralization of calcium carbonate, matrix proteins and other organic matrixes. Inorganic component counts for about 95% weight of all, organic matrix like protein and polysaccharide contributes to about 5%. There are three polymorphs of calcium carbonate, which are calcite, aragonite and vaterite, respectively. Calcite is the most thermodynamically stable polymorph, while vaterite and aragonite are thermodynamically unstable and can transform easily to calcite [4–7]. Therefore, vaterite is quite scarce in nature as natural mineral. But in biologic system, vaterite and aragonite can nucleate and grow stably, and their crystal lattice can maintain steadily with the existence of organic matrix in microenvironment precipitated by living cells [4, 8]. Therefore, the identification of matrix proteins in pearls, especially some important trace matrix proteins is a crucial problem to further understand biomineralization mechanism.

The researchers have extracted and purified various matrix proteins from nacreous layer of mollusks [9–14]. These matrix proteins are extracted by EDTA or weak acid (acetic acid) by means of dissolving calcium carbonate crystals in nacreous layer. On the basis of their dissolubility in the extraction fluid, the matrix proteins are divided into soluble organic matrix and insoluble organic matrix [15]. However, the formation of nacre is a complex process that requires a variety of proteins synergy. Although some types of matrix proteins have been identified and their effects on nacre formation have also been studied, it is still not enough for the understanding of biomineralization mechanism.

Aragonite crystals and vaterite crystals are often co-existed in the pearls. What is the difference between matrix protein extracted from aragonite pearls and vaterite pearls? Is the difference of matrix protein a reason leading to aragonite/vaterite transformation? To solve these problems, water and weak acid were used to extract water soluble matrix (WSM), acid soluble matrix (ASM) and acid insoluble matrix (AIM) from aragonite pearls and vaterite pearls to analyze the micro-structural characteristics and the differences in composition of matrix proteins. As much as we know, there is no report about the characterization of organic matrix extracted from vaterite pearls and comparison of organic matrix from aragonite pearls with that from vaterite pearls.

## 2. Materials and methods

### 2.1. Extraction of three kinds of matrix proteins from aragonite pearls and vaterite pearls

Aragonite pearls and vaterite pearls were both originated from *Hyriopsis cumingii* in Zhuji (Zhejiang province, China). These pearls were fine selected to make sure that aragonite pearls did not include vaterite crystals and vaterite pearls were composed of almost pure vaterite crystals. These two kinds of pearls were washed, alcohol-

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soaked for 10 h and then air dried. The samples were fine ground in the agate mortar, and then the pearl powders were sieved to make the particle size smaller than 50 μm. 50 g aragonite pearl powder and vaterite pearl powder were prepared for the experiment. These powders were washed with deionized water, centrifugalized and dried.

According to the previous study, a peculiar method was exploited to extract WSM, ASM and AIM from aragonite pearls and vaterite pearls, respectively [16, 17]. Specific extraction process of the matrix proteins was displayed in Fig. 1.

### 2.2. Characterization

#### 2.2.1. SDS-PAGE electrophoresis

Separation of matrix protein components and preliminary measurement of molecular weight were carried out by the method of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, DYY-III electrophoresis meter, BEIJING LIUYI Instrument Factory). 10 μl matrix protein solutions with the concentration of 10 μg/mL were used for the electrophoresis analysis. Samples were mixed with an equal volume of 2× electrophoresis sample buffer (0.125 M Tris–HCl, 4% SDS; 20% v/v glycerol, 0.2 M DTT, 0.02% bromophenol blue, pH 6.8), and heated at 95 °C for 4 min, and then analyzed by SDS-PAGE using 15% acrylamide resolving gels. The stacking gel was 4% acrylamide (Bio-Rad). The gels were fixed with a mixture of ethanol, acetic acid, and deionised water (40:10:50) for 1 h. After washing in water for 5 min, the gels were stained with Coomassie Brillant Blue R250 (0.1% in 25% methanol, 10% acetic acid). Molecular weights were estimated by comparison with the migration rates of standard protein.

#### 2.2.2. X-ray diffraction

X-ray diffractometer (Rigaku Company) was used to determine the inorganic phase of the pearls and the structure of matrix proteins with 2-theta ranging from 0 to 60° (Minimum Step of 2θ: 0.002°).

#### 2.2.3. Fourier transform infrared spectroscopy

Amide peaks of matrix proteins were measured with KBr pellet by Fourier transform infrared spectrometer (Perkin-Elmer Company) between 400 and 4000 $cm^{-1}$ with a resolution of 2 $cm^{-1}$. The pellets were prepared by completely mixing potassium bromide and matrix proteins.

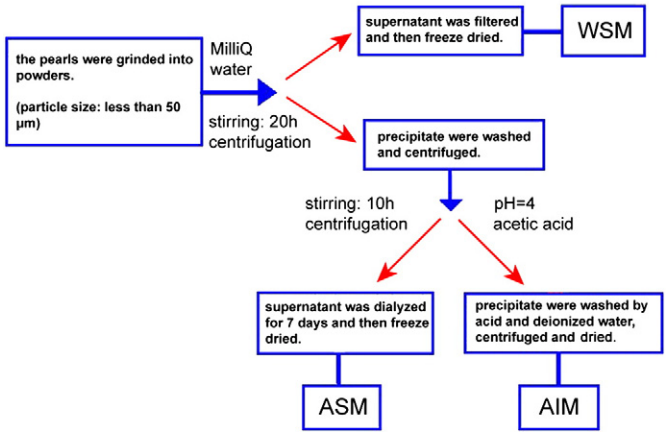


**Fig. 1.** Extraction process of water soluble matrix, acid soluble matrix and acid insoluble matrix of fresh water pearls.

## 3. Results and discussion

### 3.1. Polymorphs of aragonite pearls and vaterite pearls

Fig. 2 depicts the typical XRD patterns of selected aragonite and vaterite pearls. It can be seen in the figure that the two patterns are different The diffraction peaks are in agreement with the JCPDS standard. The characteristic diffraction peaks at 2θ of 26.2°, 33.2° and 52.6° correspond to (111), (012) and (113) crystallographic planes of aragonite respectively. The diffraction peaks at 21.1°, 27.2° and 32.9° correspond to (002), (101) and (102) crystallographic planes of vaterite respectively. The XRD results indicate that the aragonite and vaterite pearls for the experiments contain almost pure crystal phase.

### 3.2. Extraction of the matrix proteins from aragonite pearls and vaterite pearls

A fundamental information about the amount of each matrix protein extracted from aragonite pearls and vaterite pearls are listed in Table 1. Organic matrix contributing about 5% weight has been reported in many literatures and 5% should be the maximum theoretical yield. In fact, only a small proportion of the organic matrix can be extracted. That is to say, a large proportion of organic matrix is lost during the extraction process. Laurent Bédouet et al. [18] reported 0.24% (w/w) water soluble matrix was extracted from the nacreous layer of *Pinctada margaritifera*. Therefore, in this study, enough pearl

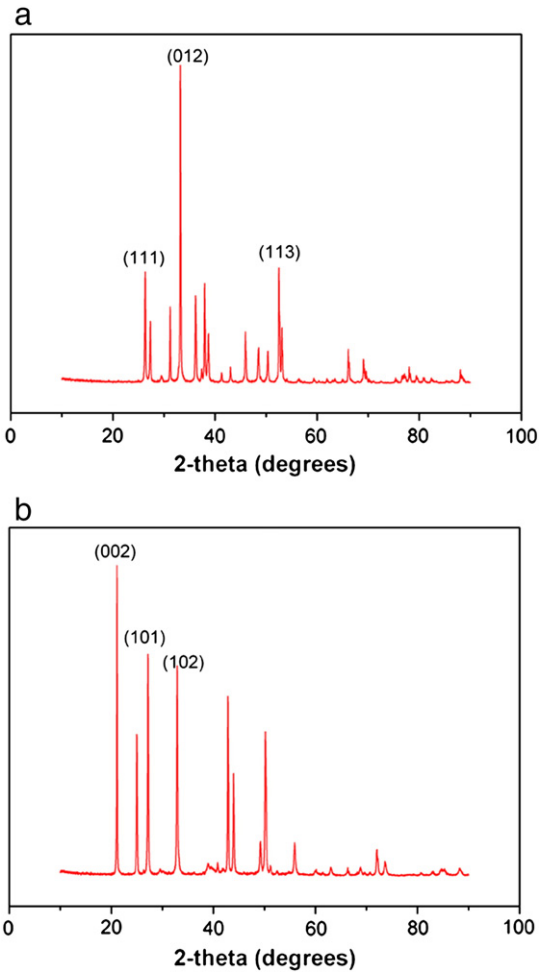


**Fig. 2.** Powder XRD results of the fresh water pearls. (a) aragonite pearls (b) vaterite pearls. The results indicate the selected aragonite pearls and vaterite pearls are composed of almost pure aragonite and vaterite respectively.