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Cloning and Characterization of an mRNA Encoding F_1-ATPase Beta-Subunit Abundant in Epithelial Cells of Mantle and Gill of Pearl Oyster, *Pinctada fucata*[*]

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Abstract: In oyster biomineralization, large amounts of calcium are absorbed from external media, transported to the mineralization site, and finally deposited via a matrix-mediated process. All these activities are very energy intensive; therefore, investigations of the energy metabolism pathways of different oyster tissues will facilitate understanding of oyster biomineralization physiology. A full-length cDNA encoding the F_1-ATPase beta-subunit (the F_1-β-subunit, a major calalytic subunit of F-ATPase) from the pearl oyster (*Pinctada fucata*) was cloned using the homology strategy with a pair of degenerated primers based on the conserved regions of other animals' F_1-β-subunit genes. Sequencing and structural analyses showed that the obtained sequence shared high identity with other animals' F_1-β-subunits, and had a unique phosphorylation site of PKC and CK II on the external surface of the putative protein. Results from semi-quantitative reverse transcription-polymerase chain reaction and *in situ* hybridization demonstrated this oyster F_1-β-subunit mRNA is abundant in the gill and mantle, and distributed widely in the periostracal groove, the outer folder, and the dorsal region of the mantle and in the gill epithelial cells. These tissues were the main regions that participate in biomineralization processes such as calcium uptake, transport, and matrix secretion. The results indicate that tissues involved in biomineralization have stronger energy metabolic processes and that F_1-ATPase might play an important role in oyster biomineralization by providing energy transport.

Key words: biomineralization; F_1-β-subunit; mantle; gill; *Pinctada fucata*

Introduction

The mollusk shell is a biomineralization product consisting of calcium carbonate crystals, matrix proteins, and other biopolymers. In pearl oysters, large amounts of calcium ions are continuously deposited onto the precisely self-assembled organic matrices[1,2], with the matrix proteins controlling the polymorph, the size and shapes of the $CaCO_3$ crystal, and finally, the shell texture[3-5]. In the past decade, many efforts have been made to study the highly controlled oyster biomineralization process. Calcium metabolism is regarded as a central physiological activity. Although the details still remain unknown, it is widely recognized that the gill and mantle play important roles in calcium metabolism[2,6-8].

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The mollusk gill is mainly responsible for calcium uptake with the mantle responsible for calcium deposition. Moreover, as the most important structure of the extrapallial space, a special compartment for shell formation, the mantle is also in charge of maintaining the unique microenvironment required for the crystal formation and growth[9,10]. Three major energy-consuming processes that must be operative in the mantle tissues[11] are ion transport to the mineralization site, elimination of H^+ resulting from the $CaCO_3$ formation, and matrix protein synthesis and secretion to the extrapallial fluid. All these activities are highly energy-intensive, with the Ca^{2+} and H^+ transport regarded as the most important processes. The ATPase molecules, membrane-bound ion transporters that couple energy production and consumption, play important roles in energy metabolism and ion transport.

The ATPases are classified as F-, V-, A-, P-, and E-ATPases based on their functional differences. The ATPases catalyse the ATP synthesis reaction by utilizing the ion gradient or hydrolyzing ATP to create an ion gradient. F-ATPase catalyses the formation of ATP by using the H^+ gradient. It contains a soluble fraction F_1, consisting of 5 subunits, with a stoichiometry of $3\alpha:3\beta:1\gamma:1\delta:1\varepsilon$ and a membrane-embedded fraction F_0[12,13]. The F_1 portion (also called F_1-ATPase) catalyzes ATP synthesis using the proton flow through F_O via the binding change mechanism and hydrolyzes the ATP molecule as an ATPase. Extensive study of F_1-ATPase have demonstrated that the beta subunit (F_1-β-subunit) is the main catalytic subunit[14,15] and the possible regulative domain of F_1-ATPase. In particular, the F_1-β-subunit has been specified as a calcium-binding protein which might introduce new possibilities for calcium-dependent regulation of ATP synthesis[16,17]. Recent work revealed that the F-ATPase is sited on the epithelium cell surface in some kinds of cells[18-20], as well as in the mitochondria inner membrane. This type of F-ATPase is active in ATP synthesis and can trigger unusual physiological pathways such as cell proliferation and migration[21-23]. All these interesting discoveries have demonstrated that the F_1-β-subunit participates in a wide range of physiological activities.

This study describes the cloning and characterization of the mRNA encoding the *Pinctada fucata* F_1-β-subunit (pfATPB). This is the first complete coding sequence of the F_1-β-subunit identified in the bivalves. Structural analysis identified some new features of this protein. Spatial distribution and expression studies of pfATPB provided useful information describing the metabolic states of various oyster tissues, with indicating that F-ATPase is required for the energy supply to biomineralization activities.

1 Materials and Methods

1.1 Total RNA extraction

Adult pearl oysters *P. fucata* were obtained from the Guofa Pearl Farm in Beihai, Guangxi Province, China. Tissues including mantle, gill, digestion gland, and gonad were isolated and kept in RNAlater (Ambion, Austin, USA). Total RNA was extracted from 200 mg tissues using TRIzol solution (Invitrogen, CA, USA) according to the manufacturer's specifications. The RNA samples were resuspended and quantified spectrophotometrically at 260 nm with an Utrospec 3000 UV/Visible Spectrophotometer (Amersham, Netherlands). The RNA integrity was determined by fractionation on 1.0% formaldehyde-denatured agarose gel stained with 1 μg/mL ethidium bromide.

1.2 Reverse transcription-polymerase chain reaction (RT-PCR)

RT-PCR was applied to obtain the cDNA fragment of the oyster F_1-β-subunit gene from the mantle. A pair of degenerate oligonucleotide primers was synthesized based on the conserved amino acid sequences of the F_1-β-subunit. The FBF1 (5′-GCC AA(G/A) GC(T/C) CAT GG(T/A) GG(A/T)TAC TC-3′) primer corresponds to the amino acid sequence (AKAHGGYS) while the FBR1 (5′-TCA TCC ATA CCC A(A/G)(A/G) ATG GCA-3′) primer corresponds to the antisense strand of the sequence encoding (AILGMDE). 2.5 μg of total mantle RNA was transcribed into the cDNA using the Superscript II and oligo (dT) primers (Invitrogen, CA, USA). The PCR reaction was performed with denaturation at 95℃ for 5 min, followed by 30 cycles of 95℃ for 1 min, 51℃ for 45 s and 72℃ for 1 min, with the final extension at 72℃ for 7 min. PCR products of the expected size (660 bp) were excised and purified with the Wizard PCR Prep DNA purification system (Promega, Madison, USA). The purified PCR products were then subcloned into pGEM-T easy