Available online at www.sciencedirect.com
SCIENCE DIRECT
Comparative Biochemistry and Physiology, Part B 144 (2006) 254–262
CBP
www.elsevier.com/locate/cbpb

Shematrin: A family of glycine-rich structural proteins in the shell of the pearl oyster *Pinctada fucata*

Masato Yano [a], Kouhei Nagai [a], Koichi Morimoto [a,b], Hiroshi Miyamoto [a,c,*]

[a] *Wakayama Prefecture Collaboration of Regional Entities for the Advancement of Technological Excellence, Japan Science and Technology Agency, Japan*
[b] *Department of Biotechnological Science, School of Biology-Oriented Science and Technology, Kinki University, Nishimitani, Kinokawa, Wakayama 649-6493, Japan*
[c] *Department of Genetic Engineering, School of Biology-Oriented Science and Technology, Kinki University, Nishimitani, Kinokawa, Wakayama 649-6493, Japan*

Received 19 January 2006; received in revised form 10 March 2006; accepted 10 March 2006
Available online 16 March 2006

Abstract

Random sequencing of molecules from a cDNA library constructed from mantle mRNA of the pearl oyster *Pinctada fucata* was used to obtain information on organic matrix proteins in the shell. In the determined sequences, we identified 7 distinct cDNAs encoding similar glycine-rich domains. Complete sequence analysis of these cDNAs showed that the predicted sequences of the proteins, which we named shematrins, possessed similar domains comprising repeat sequences of two or more glycines, followed by a hydrophobic amino acid. In addition, in shematrin-1, -2 and -3, a repeat domain designated as XG_nX (where X is a hydrophobic amino acid) was conserved. It is of further note that all the shematrin proteins have RKKKY, RRKKY or RRRKY as their C-terminal sequence. According to northern blot analysis, all shematrins are exclusively expressed in the mantle, and particularly in the edge region of the mantle; furthermore, peptide fragments similar to shematrin-1 and -2 were detected in the prismatic layer of shells by MALDI-TOF/TOF MS analysis. These findings suggest that many of shematrins are synthesized in the mantle edge and secreted into the prismatic layer of the shell, where the protein family is thought to provide a framework for calcification.

Keywords: Mollusc; Pearl oyster; Shell; Calcification; Mantle; Glycine-rich protein; Framework; Prismatic layer

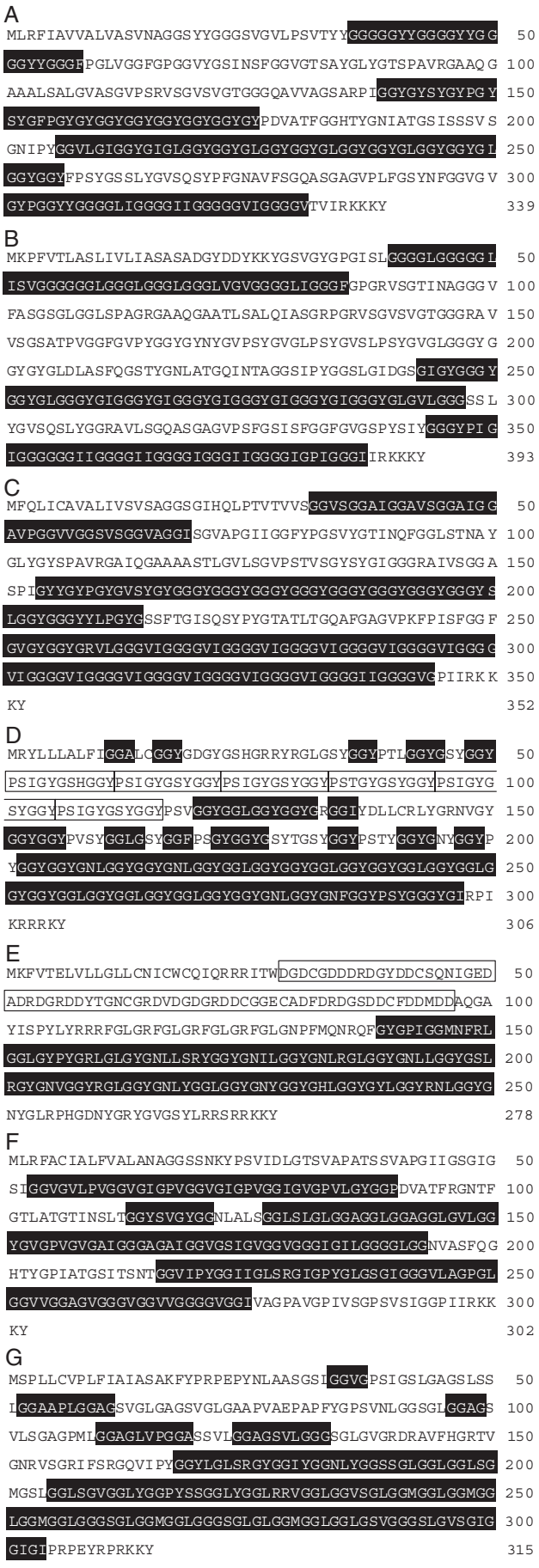
1. Introduction

In molluscs, the shell is a distinct calcified structure responsible for protection of the soft body. The characteristic structural organization of the shell is of interest, and an understanding of the enormous variation in shell morphology in different species of mollusc, the genes that regulate the process of calcification, and the construction of calcified hard tissues in invertebrates has been sought by many investigators. In many mollusc shells, several layers are observed: the prismatic layer, which consists of polygonal crystals; the foliate structure, which comprises parallel crystalline sheets; the crossed lamellar structure, which comprises lamellae composed of mutually parallel crystals; and the nacreous layer, which consists of tablets stacked in a brick-wall pattern (Wilbur, 1964; Bevelander and Nakahara, 1969; Wise, 1970; Nakahara, 1991; Hedegaard and Wenk, 1998). In the pearl oyster, the shell has an external periostracal layer and an inner calcareous layer, which is composed of the prismatic layer covering the outer surface of the shell and the nacreous layer facing the body of the pearl oyster (Lowenstam and Weiner, 1989). In many molluscs, including the pearl oyster, these characteristic shell structures are made of precipitated calcium carbonate ($CaCO_3$), which is present in aragonite and calcite crystalline forms in the nacreous layer and the prismatic layer, respectively (Wilt et al., 2003). The determination of crystal type (aragonite or calcite) and the formation of microstructures such as the prismatic and the nacreous layers are thought to be regulated by protein components (so-called organic matrix proteins) which are present in shells (Belcher et al., 1996; Falini et al., 1996).

Molecular biology techniques have been successfully applied to shell matrix proteins to isolate and sequence cDNAs. The aragonitic nacreous layer of mother-of-pearl has been used to

* Corresponding author. Department of Genetic Engineering, School of Biology-Oriented Science and Technology, Kinki University, Nishimitani, Kinokawa, Wakayama 649-6493, Japan. Tel.: +81 736 77 3888; fax: +81 736 77 4754.
E-mail address: miyamoto@waka.kindai.ac.jp (H. Miyamoto).

1096-4959/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpb.2006.03.004



isolate organic matrix proteins to study the mechanisms underlying luster formation in a living organism. MSI60 (Sudo et al., 1997), pearlin (N16) (Samata et al., 1999; Kono et al., 2000; Miyashita et al., 2000), lustrin A (Shen et al., 1997), mucoperlin (Marin et al., 2000), perlucin (Weiss et al., 2000; Mann et al., 2000) and perlustrin (Weiss et al., 2000, 2001) have been characterized as proteins found in nacre. Nacrein, which is similar to carbonic anhydrase of many organisms and has a glycine- and asparagine-repeat domain, seems to be translocated to both of the prismatic and the nacreous layer, where it is thought to regulate mineralization of $CaCO_3$ (Miyamoto et al., 1996, 2003, 2005). MSI31 (Sudo et al., 1997), prismalin-14 (Suzuki et al., 2004) and aspein (Tsukamoto et al., 2004) have been identified as proteins involved in formation of the prismatic layer. Aspein is an acidic protein that has a sequence rich in aspartic acid and is expressed at the outer edge of the mantle. MSI31 contains Gly-rich sequences such as GGGGL and GGY and is thought to function as a framework protein, while prismalin-14 also contains GGY sequences and has been shown to be expressed in the outer fold of the mantle.

Organic matrix proteins are produced in the mantle, which is a thin tissue surrounding the internal organs of molluscs. Proteins produced in the mantle are secreted into the extrapallial space, where calcium carbonate is crystallized to construct unusual microstructures. The mechanism of this process is unknown, but may involve interactions of the matrix proteins and inorganic ions present in the extrapallial space, leading to crystallization of $CaCO_3$ and morphogenesis of the species-specific appearance of the shell. As a conserved organ involved in shell formation in the invertebrate mollusc, the mantle is particularly suitable for analyzing the process of mineralization by living organisms, and it is also easy to handle. However, despite major advances in the understanding of the sequences and biochemistry of matrix proteins obtained from the mantle in molluscs, information on proteins found in shells following secretion from the mantle is limited, and little is known about the mechanisms through which diverse calcified morphologies are made. To address this problem and accelerate the accumulation of information on matrix proteins, we constructed a cDNA library from poly(A)$^+$ RNA isolated from the mantle of the pearl oyster *Pinctada fucata*, and conducted a search for sequences rich in glycine. This search identified several genes that are potentially involved in mantle function and formation of the mineralized shell. The predicted protein products of these novel genes that we named shematrins (*she*ll *matr*ix protei*n*), contain common glycine-repeat sequences, which are also similar to those found in MSI31 and prismalin-14.

Fig. 1. Predicted amino acid sequences of shematrin. A cDNA library was constructed from poly(A)$^+$ RNA isolated from the mantle of the pearl oyster *P. fucata* and subjected to sequencing of cDNAs randomly isolated from the cDNA library. The shematrin cDNAs encoding glycine-repeat sequences were selected, and their entire nucleotide sequences were determined. Because the cDNAs of shematrin-3 and -6 were not full length, we carried out 5′ RACE to know the entire sequence of the full-length cDNAs. In all shematrin, glycine-repeat sequences are highlighted. (A) Shematrin-1. (B) Shematrin-2. (C) Shematrin-3. (D) Shematrin-4. The repeat domain composed of PSIGYGSYGGY is boxed. (E) Shematrin-5. The acidic domain is boxed. (F) Shematrin-6. (G) Shematrin-7.