Primary structure of a soluble matrix protein of scallop shell: Implications for calcium carbonate biomineralization

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ABSTRACT

Soluble proteins in the scallop (Patinopecten yessoensis) foliated calcite shell layer were characterized using biochemical and molecular biological techniques. SDS PAGE of these molecules revealed three major protein bands, 97 kD, 72 kD, and 49 kD in molecular weight, when stained with Coomassie Brilliant Blue. Periodic Acid Schiff staining and Stains-All staining indicated that these proteins are slightly glycosylated and may have cation-binding potential. N-terminal sequencing of the three proteins revealed that all three share the same amino acid sequence at least for the first 20 residues. A partial amino acid sequence of 436 amino acids of one of these proteins (MSP-1) was deduced by characterization of the complementary DNA encoding the protein. The deduced sequence is composed of a high proportion of Ser (31%), Gly (25%), and Asp (20%), typifying an acidic glycoprotein of mineralized tissues. The protein has a basic domain near the N-terminus and two highly conserved Asp-rich domains interspersed in three Ser and Gly-rich regions. In contrast with prevalent expectations, (Asp-Gly)n-, (Asp-Ser)n-, and (Asp-Gly-X-Gly-X-Gly)n-type sequence motifs do not exist in the Asp-rich domains, demanding revision of previous theories of protein-mineral interactions.

INTRODUCTION

Minerals produced by organisms often have crystal shapes clearly different from those formed inorganically. Most such biominerals are a composite of inorganic crystals and organic molecules such as lipids, polysaccharides, and proteins, collectively known as the organic matrix. It is generally postulated that the elaborate fabrication of biominerals arises from specific molecular interactions at inorganic-organic interfaces (Mann et al. 1993), and that the organic matrix represents many of the important molecules involved in the interactions controlling crystal growth (e.g., Watabe and Wilbur 1960; Lowenstam 1981; Weiner 1984; Lowenstam and Weiner 1989).

Calcium carbonate is one of the most common biominerals, and its matrix molecules, especially of molluscan shells, have been studied to a considerable extent to unveil their roles in the mineralization processes. The matrix molecules have been classified conventionally into two types based on their solubility in aqueous solutions: the insoluble matrix is thought to be largely intercrystalline (Krampitz 1982) and provides a framework where mineralization is to occur, whereas the soluble matrix is known as intracrystalline or located on the intercrystalline matrix surfaces, but its functions are still poorly understood (Addadi and Weiner 1997).

Advocated functions of the mainly proteinaceous, soluble matrix of the molluscan shell in particular, include:

(1) induction of oriented nucleation (Weiner 1975; Weiner and Addadi 1991); (2) inhibition of crystal growth (Wheeler et al. 1981; Wheeler 1992); (3) control of aragonite-calcite polymorphism (Falini et al. 1996; Belcher et al. 1996); and (4) enhancement of mechanical properties of the crystals (Berman et al. 1988; Berman et al. 1990). Most of the evidence to support these hypotheses has been obtained through in vitro experiments. The weakness of these studies is that unpurified proteins or protein fractions of dubious homogeneity have been applied in the biochemical analyses and in the in vitro mineralization experiments, and that the stereochemical relationships between the organic and inorganic phases have been presumed without precise information of the fine structures of the proteins.

To understand the underlying mechanisms of the protein-mineral interactions, it seems essential first of all to know the primary structure of the proteins involved. However, only a limited number of amino acid sequences have been determined so far for the calcium carbonate matrix proteins. The available sequences comprise those from spicules of sea urchin emryo (Sucov et al. 1987; Katoh-Fukui et al. 1991; Katoh-Fukui et al. 1992; Benson and Wilt 1992), from pearl oyster shell layers (Miyamoto et al. 1996; Sudo et al. 1997), and from the nacre of a gastropod shell (Shen et al. 1997), in addition to partial sequences from brachiopod shells (Cusack et al. 1992), an oyster shell (Wheeler 1992), and gastrolith of a crayfish (Ishii et al. 1996; Ishii et al. 1998). Here we present a partial amino acid sequence of the molluscan shell pro-

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