Proteomic analysis of shell matrix proteins in the pond snail *Lymnaea stagnalis*: identification of potentially functional proteins

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Matrix proteins have important roles in molluscan shell formation, and their amino acid sequences have been characterized for some species. However, the mechanisms of shell formation have barely been clarified. In order to setup a platform for a systematic functional analysis of shell matrix proteins, we performed a combined transcriptome and proteomic analysis of the shell matrix proteins for the pond snail *Lymnaea stagnalis*. We found a total of 203 shell matrix proteins from the shell matrix of *L. stagnalis*. A total of 161 amino acid sequences of them showed sequence similarities to known proteins, including four paralogs of dermatopontin, which was previously reported from the shell matrix of *L. stagnalis*, when searched against public databases, while the remaining 42 showed no similarity to the proteins in the current databases. Next, in order to discriminate 'functional' shell matrix proteins from those that were accidentally entombed in the shells, we compared the levels of expression of these shell matrix proteins between the right side and the left side of the mantle, which makes the shell, underlying assumption being that genuine functional shell matrix protein genes would be more strongly expressed in the right hand side of the mantle in the dextral shells, while there would be no such differential expression pattern for the proteins which were accidentally trapped within the shells. Actin is the most abundant shell matrix protein found in the shell of *L. stagnalis*, but the expression patterns of the actin gene showed no difference between right and left of the mantle. On the other hand, the second most abundant shell matrix protein called Pif-like protein, which is an acidic shell matrix protein identified also in *Pinctada fucata* and *Crassostrea gigas*, showed that its gene is more strongly expressed in the right hand side than the left hand side of the mantle. Our results suggest that Pif-like protein is a functional shell matrix protein, while actin is a protein occluded in the shell accidentally. Finally, we searched for conserved domains of these amino acid sequences. We found various domains and classified them into six categories. Those are extracellular protein, enzyme, cation-interaction protein, polysaccharide interaction protein, proteinase inhibitor, and others. Conserved domains allow us to estimate possible functions of novel shell matrix proteins. Characterization of those novel proteins, and functional analyses of all those proteins identified in this study for this 'model organism' will help understand the mechanisms of biomineralization as well as the evolutionary processes of shell formation in molluscs.

Keywords: Transcriptome, Proteomic analysis, Biominelarization, Shell formation, Matrix protein

