

Proteomic analysis of shell matrix proteins in the pond snail *Lymnaea stagnalis*: discrimination of potentially functional proteins from accidentally occluded proteins

*Akito Ishikawa¹, Keisuke Shimizu², Yukinobu Isowa³, Takeshi Takeuchi⁴, Keiji Kito³, Manabu Fujie⁴, Michinari Sunamura¹, Nori Satoh⁴, Kazuyoshi Endo¹

1. University of Tokyo, Graduate School of Science, Department of Earth and Planetary Science, 2. Japan Agency for Marine-Earth Science and Technology, 3. Meiji University, School of Agriculture, 4. Okinawa Institute of Science and Technology Graduate University

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 207 shell matrix proteins from the shell matrix of *L. stagnalis*. A total of 165 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*, while the remaining 42 showed no similarity to the proteins in the current databases. In order to discriminate functional shell matrix proteins from those that were accidentally buried 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 tissue which makes the shell. Underlying assumption is that genuine functional shell matrix protein genes would be more strongly expressed in the right hand side of the mantle in the dextral shell, while there would be no such differential expression pattern for the proteins which were accidentally trapped within the shells. Our results suggest that Pif-like protein is a functional shell matrix protein, while actin is a protein trapped within the shell accidentally. Comparisons of the expression patterns between the mantle and the foot tissues indicated that a total of 29 genes are expressed specifically in the mantle tissue with 25 out of them being expressed stronger in the right hand side than in the left hand side of the mantle tissue. Principle component analysis of the gene expression data showed that, those supposed functional shell matrix proteins are distinguished from the other shell matrix proteins, which were possibly accidentally entombed within the shells.

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