# Molecular Evolution of the Shell Matrix Protein Aspein in Pterioid Bivalves

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Aspein is one of the unusually acidic molluscan shell matrix proteins identified from mantle tissues of the pearl oyster *Pinctada fucata*. Aspein is believed to have important roles in the specific calcite formation in the prismatic layer. In this study, we identified three homologues of Aspein in the congeneric species *P. maxima*, and named them PmAspein-1, 2 and 3. They all have a poly-Asp repeat domain (D-domain) like the sequence of *P. fucata* (PfAspein) and share exactly the same N-terminal sequence. The sequence of PmAspein-3 is the most similar to that of PfAspein among PmAspeins. The results of immunoassay showed that Aspein exists only in the calcitic prismatic shell layer in the *P. maxima*, and the results of RT-PCR showed that the transcript of PmAspein-3 is expressed only in the outer edge of mantle, corresponding to the calcitic prismatic layer. The N-terminal sequences of PmAspein-3 and PfAspein showed high sequence similarities to each other, suggesting that they are functionally important. In PmAspein-3, the D domain, which is likely to have $Ca^{2+}$ binding capacity, is shorter than that of P. fucata, suggesting that a long D domain is not required for calcite shell formation. The C-terminal region of PmAspein-1 and 2 is not similar to that of PfAspein but is similar to MSI60 and MSI25, respectively, both of which are known in P. fucata as shell matrix proteins in the aragonitic nacreous layer. The phylogenetic tree showed that PmAspein-1, 2 and 3 became differentiated after the separation of *P. maxima* and *P. fucata*. The evolutionary rates of Aspeins are lower than Shematrins which are insoluble matrix protein in the prismatic layer, suggesting that the functional constraints are generally higher in Aspein than in Shematrin.