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Molecular evolution of the shell matrix protein Aspein in pterioid bivalves

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Acidic shell matrix proteins are likely to have many important functions for shell precipitation. Aspein is one of the unusually acidic shell matrix proteins identified from the mantle tissue of the pearl oyster *Pinctada fucata*. Aspein is inferred to have important roles in the calcite formation in the prismatic layer. In this study, we identified Aspein homologues from a congeneric species *Pinctada maxima*, as well as from two closely related pterioid species *Isognomon perna* and *Pteria penguin*. The results of immunoassay showed that they exist in the calcitic prismatic shell layer but not in the aragonitic nacreous shell layer. The SEP (Ser-Glu-Pro) motif and the DA (Asp-Ala) repeat motif were conserved among these Aspeins, suggesting that those motifs are functionally important. The high proportion of Asp and Gly in D domain, which is believed to have Ca²⁺ binding capacity, is also conserved, suggesting that this feature is important for the function of D domain. However, other features of the primary structure of Aspeins showed a significantly high level of variation among very closely related species, suggesting that any specific sequences as template for nucleation are not required for the function of acidic shell matrix proteins.