

Shell-muscle attachment in the bivalves *Ostrea stentina* Payraudeau, 1826 and *Anomia ephippium* Linnaeus, 1758

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The muscles of the mantle in bivalves attach to the shell valves and retract the mantle edges. Among them, the adductors are particularly important for the survival of the animal. Most studies have addressed the type and morphology of the muscular fibers and filaments, and a few have focused on the attachment of these muscles to the shell. However, the mechanism of transport through the muscle to the myostracum was never addressed. The goal of this research is to describe ultrastructure and the transport of material across the adductor muscle-myostracum attachment in *Ostrea stentina* and *Anomia ephippium*.

We examined 10 specimens of *O. stentina* and 10 specimens of *A. ephippium*, collected in the littoral of Málaga (south Spain). Specimens were fixed in 2.5% glutaraldehyde (4 °C), decalcified in 2% EDTA, post-fixed in OsO₄ (2%) and embedded in epoxy resin Epon 812 (EMS). Samples for calcium detection were post-fixed in a mixture of OsO₄ (2%) and potassium hexahydroxoantimonate (2%) in PBS. Ultra-thin sections (50 nm) were stained with uranyl acetate (2%).

The adductor muscles in both species are composed of “smooth” and “striated” muscles. The outer epithelium of the mantle continues across the adductor muscle area and their cells contain many vesicles. Bundles of filaments connect the muscular cells with the extrapallial space through hemidesmosomes. The extrapallial space (ca.100-150 nm thick) is filled with organic secretions from the vesicles of the mantle cells. Additional bundles of microfilaments of collagen fibers cross the extrapallial sheet, from the hemidesmosomes to inside the myostracum, where they form a network. EDX analysis shows the presence of calcium inside vesicles from the mantle cells, the extrapallial sheet and the myostracum network. The presence of a layer of cells between the muscular cells and the shell was confirmed the existence of this cell layer, which is the continuation of the outer mantle epithelium across the adductor muscle. The extrapallial space is replenished with secretions of the mantle cells and collagen fibers, seemingly originated in the hemidesmosomes. The presence of calcium inside some vesicles suggests that part of the calcium is transported thereby. It would be interesting to study whether calcium is transported as amorphous calcium carbonate or as a crystalline phase.