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PS-3 The Carbonic anhydrase in calcified endoskeleton: Novel activity in bio-calcification in alcyonarian

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Carbonic anhydrase (CA) is a key enzyme in chemical reaction of living organism and has been found to be associated with calcification in a number of invertebrates including calcareous sponges, but until now no direct evidence has been advanced to show CA activity in alcyonarian corals. However, it is essential to understand the role of CA in the process of bio-calcification in alcyonarian. Here we describe the novel functions of CA and its relation to formation and degradation of calcified hard tissues in alcyonarian. We find that proteins from alcyonarian can control the morphology of CaCO_3 crystals. In an in vitro assay we show that the matrix proteins extracted from the calcitic spicules of alcyonarian induce the formation of amorphous calcium carbonate prior to its transformation into the calcitic crystalline form. We also show scanning electron micrographs of the rhombohedral calcite crystals used as template, the protein imprinted with these crystals. Soluble calcium-binding proteins, which are responsible for calcium carbonate nucleation and crystal growth, were detected as radioactive bands by ^{45}Ca autoradiography. Two Ca^{2+} -binding proteins were found to be radioactive and have CA activity that catalyzes HCO_3^- formation. We also present evidence to show that the bicarbonate generated by CA activity is combined with calcium ions to form calcium carbonate (calcite), which in turn associates with organic matrices to form a unique biomineralized structure. Our observations strongly suggest that the matrix protein in alcyonarian is not only structural protein but also a catalyst.