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Cooption of secretory phospholipase (SPLA2) for different aspects of gravity receptor-associated mineralization in vertebrate phylogeny

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Vertebrate gravity-associated minerals consists of either a single large stone (otolith), or an assembly of minute biomineral particles, otoconia ("ear dust"). Otoliths and both, amphibian and reptilian otoconia, consist of aragonite, whereas avian and mammalian otoconia consist of calcite. Vertebrate gravity-associated minerals are the product of site-directed biologically-controlled mineralization. Insoluble frame work molecules specify sites of nucleation and direction of crystal growth. Soluble matrix proteins modulate growth kinetics and crystal morphology. It is most remarkable that the principal insoluble frame work protein, otolin, is the same for both, otolith and otoconia. Otolin is a novel type of collagen, homologous to the network-forming collagen type X prevalent in mature chondrocytes. The principal soluble matrix proteins of calcitic, aragonitic, and most likely also of vateritic otoconia are all homologs of SPLA2, which is most prevalent in pancreatic secretion and snake venoms. Otonin90 (OC90), the principal soluble matrix protein of calcitic otoconia consists of two SPLA-like (SPLAL) domains, which are connected by a sizeable linker segment and contain significant terminal extensions. The MW of the protein backbone amounts to approximately 50 kDa. The molecule contains, in addition massive post-translational modifications, 80% of which are accounted for by sulfated GAGs, resulting in a total MW of 100 KDa. The protein backbone is moderately acidic, pI 4.4, but the pI of the whole molecule is 2.9, indicating a substantial acidity of the GAG component.

In adapting SPLA2 for mineral modulation the enzymatic site is modified and presumed nonfunctional. The seven SH- bonds are rigorously conserved in both, OC90 and otoconin22 (OC22). It appears that the SH-bonds of the parent SPLA2 are intended to stabilize the molecule to ensure continued enzymatic activity in the hostile environment of the gut. It therefore seems logical that SPLA2 was coopted for mineral modulation not because of its enzymatic activity but to provide a rigid interface conducive to mineral interaction.

To provide sufficient matrix protein for in vitro experimentation, we generated recombinant proteins. Circular dichroidism (CD) spectra indicate that the alpha helical structure of the parent SPLA2 is conserved in the SPLAL domains. A precedent of alpha helical structure for provision of a rigid interface was demonstrated to be essential for the activity of the antifreeze protein of the winter flounder. Support for alpha helical structure as signature property of the SPLAL domains of OC90 is the fact that rOC90, when exposed to calcium or carbonate-rich ionic solutions resulted in marked conformational changes, with the largest effects seen by combined application of both ions. The capacity to induce reproducible conformational changes is a testament to the quality and authenticity of rOC90. Alpha helical structure as signature characteristic of OC90 is contrary to the traditional paradigm of beta sheet structure as the essential agent in mineral interaction of highly acidic mollusk shell proteins. Apart from the alpha helical regions of the SPLAL domains, homology-based molecular modeling indicates that most of the linker segment and the terminal extensions consist of unordered structure. The significance of unordered structure in mineral interaction has recently been pointed out by several authors. For instance, the linker segment exhibits a 20 amino residue regions, dominated by hydrogen bonding and charged residues, in other words a hydrophilic segment, suitable for mineral interaction; the same applies to the C-terminal extension. Homology-based molecular models of the SPLAL domains exhibit a spherical surface with a uniform negative electrostatic potential that should be effective in attracting calcium.

OC22, the principal soluble aragonitic matrix protein, consists of a single SPLAL domain, with a minor N-linked glycoside. rOC22 in vitro does not induce formation of aragonite, but of calcite as default option, analogous to other aragonitic matrix proteins, e.g. AP8 alpha and beta of the avalone nacre. It has been shown that aragonitic proteins are able to express aragonite only in association with the appropriate insoluble matrix. The effects of the

protein upon calcite modification are qualitatively identical to the effects of OC90 (nucleation density, crystal size and morphologic change), but are quantitatively much less. The most unexpected SPLA2 domain-related aspect is the recent discovery of an OC90 ortholog (Otoc1) in otoliths even though OMP, a derivative of melanotransferin is the principal soluble matrix protein of otoliths. We, heretofore, assumed that OC22, as a single SPLAL domain, constituted the earliest manifestation of this cooption in vertebrate phylogeny. Significantly, the presence of Otoc1 is not incidental. Knock-down of the gene results in agenesis or malformation of otolith with a change from aragonite to calcite.

In summary, this presentation serves to illustrate the wide distribution and varied function of a coopted lipolytic enzyme in a wide range of mineralization-related contexts.