Alkaline Phosphatase and Nucleotide Pyrophosphatase/ Phosphodiesterase-1 reconstituted in liposomes as Matrix Vesicle Mimetics.

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Endochondral calcification is mediated by chondroblast- and osteoblast-derived matrix vesicles (MV). The primary function of tissue-nonspecific alkaline phosphatase (TNAP) is to degrade extracellular inorganic pyrophosphate (ePPi), a mineralization inhibitor, which is produced by the enzymatic activity of nucleotide pyrophosphatase/phosphodiesterase-1 (NPP1), restricting the concentration of ePPi, to maintain a Pi/PPi ratio permissive for normal bone mineralization. In this work we established a proteoliposome system as osteoblast-derived MVs biomimetic to study the interplay of TNAP and NPP1 during catalysis of biomineralization substrates. First, we studied the incorporation of TNAP in DPPC, DPPC:DPPS (9:1 and 8:2) and DPPC:DODAB (9:1 and 8:2) liposomes, which proved complete in DPPC liposomes. Next, either recombinant TNAP, recombinant NPP1 or both together were reconstituted in DPPC liposomes and the hydrolysis of ATP, ADP, AMP, PEA, PLP, p-NPP, pNP-TMP and PPi by these proteoliposomes was studied at physiological pH. pNP-TMP was exclusively hydrolyzed by NPP1-liposomes, whereas ATP, ADP, AMP, PEA, PLP, p-NPP and PPi were hydrolyzed both by TNAP-liposomes and TNAP+NPP1-liposomes, in some cases cooperatively. Hydrolysis of PPi by TNAP-, and TNAP+NPP1-proteoliposomes occurred with catalytic efficiencies and mild cooperativity effects to those manifested by wild-type osteoblast-derived MVs. The reconstitution of TNAP and NPP1 into proteoliposomes generates a phospholipid microenvironment that allows the kinetic study of phospho-substrate catabolism in a manner that recapitulates the native MV microenvironment. **Supported by** FAPESP, CNPq, CAPES and DE12889 and AR47908 from NIH.