Molecular Mechanisms Underlying Matrix Vesicle-Induced Mineralization During Bone Formation: an Enzyme Kinetic Approach.

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Mineralization of cartilage and bone occurs by processes that facilitate the deposition of hydroxyapatite in specific areas of the extracellular matrix, mediated by chondroblast- and osteoblast-derived matrix vesicles (MVs). The primary function of tissue-nonspecific alkaline phosphatase (TNAP) is to degrade extracellular inorganic pyrophosphate (ePP_i), a mineralization inhibitor, which is produced by nucleotide pyrophosphatase/phosphodiesterase-1 (NPP1), restricting the concentration of ePP_i, to maintain a P_i/PP_i ratio permissive for normal bone mineralization. We studied the efficiency of phospho-substrates catalysis by osteoblast-derived MVs, kinetically analyzing at pH 7.4 the hydrolysis of ATP, ADP and PP_i by isolated native wild-type (WT), PHOSPHO1 null, TNAP null and NPP1 null MVs. Comparison of the catalytic efficiencies measured for null MVs to those of native MVs identified ATP as the main substrate, hydrolyzed by WT MVs. Hydrolysis of ATP was significantly reduced in the absence of TNAP, but also of PHOSPHO1, implicating both enzymes in the processing of ATP by MVs. Deficient PP_i and ADP hydrolysis was observed in TNAP-deficient MVs underscoring that, unlike for the hydrolysis of ATP, PPi is primarily processed by TNAP. The lack of NPP1 did not affect significantly the kinetic parameters of hydrolysis for all substrates. The present study shows a strict cooperation between NPP1, TNAP and PHOSPHO1 in a MV microenvironment, enabling hydroxyapatite mineralization under conditions of restricted PP_i accumulation. **Supported by:** FAPESP, CNPq, CAPES and DE12889 and AR47908 (NIH).

Key words: alkaline phosphatase, NPP1, matrix vesicles.