Exogenous polyphosphate is not readily utilized for mineralization in vitro

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Overview

INTRODUCTION: Polyphosphates (PolyP) naturally occur in many cell types with higher concentrations in osteoblastic cells. More detailed characterization of PolyP function(s) may be pertinent to both physiological and pathological mineralization.

METHODS: We evaluated the capacity of Saos-2 and MC-3T3 bone cell lines to induce mineralization (as measured by von Kossa, Alizarin Red, and EDX) in the presence of exogenous PolyP. We also utilized ventralivellar vectors (LV) to overexpress the alkaline phosphatase (ALP) transgene in MC-3T3 cells.

RESULTS: We were unable to demonstrate PolyP-induced mineralization with either cell type, nor in MC-3T3 cells over-expressing alkaline phosphatase. We identified that Alizarin Red interacts uniquely with Ca-PolyP to produce a false positive stain.

CONCLUSION: The straightforward addition of exogenous PolyP is insufficient to induce in vitro mineralization. Care must be taken when choosing a method of mineral quantification to accurately detect Ca-P mineral, and Alizarin Red should not be used to measure mineralization in the presence of Ca-PolyP.

Mineralization Analysis: MC-3T3

MC-3T3 cells are unable to utilize PolyP as the sole phosphate source for mineral; unique Alizarin Red staining represents a false positive

Proposed Interaction of Ca-PolyP-Alizarin Red Staining

Polyphosphates (PolyPs) are inorganic chains of phosphates, which can provide a source of phosphate in the body. PolyP can also be a reservoir for calcium due to its ability to chelate large amounts of calcium.

This chelation could contribute to the initial nucleation event in mineralization: when PolyPs are metabolized, concentrations of total Ca\(^{2+}\) and PO\(_4\)^{3-} above physiological saturation would be generated locally.

Alkaline Phosphatase (ALP) is an enzyme that plays a role in mineralization in vivo, and has been shown to cleave PolyP in vitro. If PolyPs initiate or promote mineralization, then these polymers may also act as novel agents to enhance bone formation when delivered clinically.

PURPOSE: Evaluate the capacity of MC-3T3 and Saos-2 cells to sustain mineralization in the presence of exogenous polyphosphate.

Experimental Design

A) In vitro mineralization assay
- MC-3T3s were treated with 3 mM orthophosphate (Pi); 3 mM 5-glycerophosphate (G5P) or 294 µM PolyP (PolyP17, chain length: 17 PO\(_4\)^{3-}) for 21 days
- Saos-2 cells (having a significantly higher endogenous ALP expression than MC-3T3s) were treated with 5 mM Pi, 5 mM G5P or 294 µM PolyP17 for 8 days
- MC-3T3 cells overexpressing the ALP transgene (generated using lentiviral vectors) were treated with 3 mM Pi, 3 mM G5P or 294 µM PolyP17 for 21 days. ALP overexpression was confirmed visually with Fast Blue staining and spectrophotometrically.

B) Mineral Analysis
- Cells were stained with Alizarin Red (identifies Ca\(^{2+}\)) or von Kossa (Pi\(^{4+}\))
- Cells were processed for electron microscopy

Mineralization Analysis: Saos-2

High concentrations of PolyP prevent G5P-mediated mineralization by Saos-2 cells; but not low PolyP concentrations

Saos-2 cells are unable to utilize PolyP as the sole phosphate source for mineralization

Electron Microscopy Analysis

Electron microscopy corroborates Alizarin Red staining and demonstrates the presence of residual PolyP after long-term culture of MC-3T3 cells

Discussion & Conclusions

- Under standard cell culture conditions, neither Saos-2 nor MC-3T3 cells were able to utilize exogenous PolyP as the sole source of phosphate to produce mineral.
- The ineffectiveness of high levels of ALP (either endogenous or overexpressed) in the metabolism of PolyP leading to mineral deposition indicates that PolyP is likely the primary enzyme implicated in PolyP metabolism or (B) optimal PolyP processing by ALP requires more conducive conditions.
- PolyP studies reveal conflicting observations: PolyP is reported as stimulating bone mineralization\(^{[1,2]}\) as well as inhibiting (pathological) mineralization\(^{[3,4]}\). Our results diverge from the former observations and align more with the latter.
- Such discrepancies surrounding the role of PolyP in mineralization warrant more systematic and comprehensive analyses of both intra- and extra-cellular PolyP, particularly given the complex interactions between PolyP and divalent cations (Ca\(^{2+}\)).
- Better understanding of the initial mechanism(s) of mineral nucleation, including the potential role of PolyPs, may lead to novel therapeutic approaches to treat bone affictions.

Reference:
1. Brin D. Bone 2012; 48(1):37-42. "Multiple roles of polyphosphate in mineralization: