SHORT COMMUNICATION

PiT-1 RECONSTITUTION INTO LIPOSOMES FOR BIOMINERALIZATION STUDIES

[1]Simão A.M.S., [1]Bolean M., [2]Hoylaerts M.F., [3]Millán J.L. and [1]Ciancaglini P.

Corresponding author: Pietro Ciancaglini, Ph.D., Department of Chemistry, FFCLRP-USP, Av. Bandeirantes, 3900, 14040-901, Ribeirão Preto, SP, Brazil, Tel.: +55 16 3602-3753; Fax: +55 16 3602-4838; E-mail: pietro@ffclrp.usp.br

[1]Department of Chemistry, FFCLRP-USP, Brazil; [2]the Center for Molecular and Vascular Biology, University of Leuven, Leuven, Belgium; [3]the Sanford Children's Health Research Center, Sanford-Burnham Medical Research Institute, La Jolla, CA, USA

During endochondral bone formation, chondrocytes and osteoblasts are responsible for the synthesis and mineralization of the extracellular matrix through a carefully orchestrated process, believed to initiate hydroxyapatite (HA) deposition within membrane invested matrix vesicles (MVs) and continue with bone mineral propagation onto the collagenous scaffold. Inorganic pyrophosphate (PPi), derived both from ENPP1-catalyzed production from extracellular nucleoside triphosphates and by cellular export via the ANK transporter, inhibits matrix mineralization. This inhibition is released through the action of TNAP, which hydrolyzes PPi, thus simultaneously removing the inhibitor and providing additional Pi for mineral formation. Ca2+ enters MVs via annexin channels and phosphate enters via a type III Na+-dependent phosphate transporter (PiT), and possibly others, to form apatite within MVs. Acidic phospholipids and other MV components are thought to nucleate these intravesicular nanocrystals. Subsequently, the intravesicular mineral grows beyond the confines of MVs onto a collagenous matrix aided by a number of promoters and inhibitors of calcification. HA crystals are still present in TNAP-deficient MVs, and it has been shown that the soluble MV phosphatase PHOSPHO1 is involved in intravesicular mineralization. As we strive to understand the physiological interplay between TNAP, PiT-1, ENPP1, and other important MV-associated enzymes and channeling proteins in the initiation of biomineralization, we must keep in mind the microenvironment in which these proteins function, which can have a profound effect on their biological properties, since phospholipids play an important role in the initiation of the biomineralization process. The ability of synthetic or natural vesicles to mimic the organizational structure and function of biomembranes makes these structures an advantageous and convenient experimental model to help us advanceour understanding of MV-mediated calcification. The proteoliposome system provides a means of reconstituting lipid vesicles that will function like MVs. Here we report the construction of proteoliposomes harboring PiT-1. PiT-1 protein was reconstituted into DPPC:DPPS 10% (molar ratio) liposomes (1 mg/ml of lipids) by the co-solubilization method, with a protein:lipid ratio of 3:1 (w/w) and a detergent concentration of 10 mg/ml. The detergent was removed by incubating the mixture with 200 mg/ml of Calbiosorb resin, with three incubations of 10, 30 and 60 minutes, at 4°C. The proteoliposome formed contained about 52% of protein reconstituted. Western blotting analysis of proteoliposome samples showed that PiT-1 was incorporated into the liposomes. Now, we intend to perform assays to measure sodium-dependent Pi/PPi uptake by the proteoliposomes to verify if the reconstituted protein is functional. Once built and characterized, these proteoliposomes can be added to fixed amounts of MVs, wild-type or deficient in specific enzymes, as a way of modulating their in vitro calcification properties. Those experiments would validate the use of these proteoliposomes in promoting or delaying calcification, and such an artificial nanovesicular system could also potentially prove useful for the repair/treatment of craniofacial and other skeletal defects and to facilitate the mineralization of titanium-based tooth implants.

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