Characteristics of minerals in vesicles produced by human osteoblasts hFOB 1.19 and osteosarcoma Saos-2 cells stimulated for mineralization

Agnieszka Strzelecka-Kiliszka, Łukasz Bozycki, Saida Mebarek, Rene Buchet, Slawomir Pikula

A Department of Biochemistry, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur Str., 02-093 Warsaw, Poland
B Université Lyon 1, 69622 Villeurbanne Cedex, France
C INSa de Lyon, 69622 Villeurbanne Cedex, France
D CPE Lyon, 69622 Villeurbanne Cedex, France
E ICMS CNRS UMR 5246, 69622 Villeurbanne Cedex, France

Abstract

Bone cells control initial steps of mineralization by producing extracellular matrix (ECM) proteins and releasing vesicles that trigger apatite nucleation. Using transmission electron microscopy with energy dispersive X-ray microanalysis (TEM-EDX) we compared the quality of minerals in vesicles produced by two distinct human cell lines: fetal osteoblastic hFOB 1.19 and osteosarcoma Saos-2. Both cell lines, subjected to osteogenic medium with ascorbic acid (AA) and β-glycerophosphate (β-GP), undergo the entire osteoblastic differentiation program from proliferation to mineralization, produce the ECM and spontaneously release vesicles. We observed that Saos-2 cells mineralized better than hFOB 1.19, as probed by Alizarin Red-S (AR-S) staining, tissue nonspecific alkaline phosphatase (TNAP) activity and by analyzing the composition of minerals in vesicles. Vesicles released from Saos-2 cells contained and were surrounded by more minerals than vesicles released from hFOB 1.19. In addition, there were more F and Cl substituted apatites in vesicles from hFOB 1.19 than in those from Saos-2 cells as determined by ion ratios. Saos-2 and h-FOB 1.19 cells revealed distinct mineralization profiles, indicating that the process of mineralization may proceed differently in various types of cells. Our findings suggest that TNAP activity is correlated with the relative proportions of mineral-filled vesicles and mineral-surrounded vesicles. The origin of vesicles and their properties predetermine the onset of mineralization at the cellular level.

1. Introduction

Bone is a dynamic form of connective tissue composed of three cell types (osteoblasts, osteocytes and osteoclasts), extracellular matrix (ECM), which provides tensile strength, and hydroxyapatite (HA), which grants mechanical resistance [1]. Osteoblasts, as well as chondrocytes in cartilage and odontoblasts in teeth, control initial steps of mineralization by producing ECM proteins and releasing matrix vesicles (MVs), which trigger apatite nucleation[2,3]. Soft tissues do not mineralize under physiological conditions, however ectopic calcification may occur under pathological conditions. This is initiated by cell transdifferentiation toward bone formative cells due to activation of genes associated with osteochondrogenesis [4]. The transdifferentiated mineral-competent cells are able to produce collagen matrix and to release vesicles, which leads to the formation of calcium phosphate deposits (apatites) in soft tissues [3]. Therefore, understanding the molecular mechanisms of calcification of osseous cells and chondrocytes shall provide some clues on how cells in soft tissues can trigger ectopic calcification.

Skeletal tissues are regulated by various enzymatic activities, growth factors, as well as by promoters or inhibitors of mineralization. Of crucial importance are the antagonistic actions of tissue-nonspecific alkaline phosphatase (TNAP) and ectonucleotide pyrophosphatase/phosphodiesterase I (NPP1), which control HA formation [5]. Inorganic pyrophosphate (PPi), a potent inhibitor of HA formation, is hydrolyzed by TNAP, while NPP1 hydrolyzes nucleotide triphosphates to form PPi. Indeed, the P/PPi ratio regulates HA formation [6,7] and deregulates cellular PPi production, degradation and transport, with possible pathological consequences [8]. Ion replacement in apatite crystals is a known biomedical procedure [9]. Thus, identification of minerals produced by cells is necessary to determine how the chemistry of calcium phosphate complexes may alter physiological mineralization and ectopic calcification. Apatites are calcium and phosphate containing minerals with the general crystal unit cell formula Ca_{10}(PO_{4})_{6}X_{2} where X = Cl, F, OH. This group of minerals is classified as follows [10]: fluorapatite (FA) Ca_{10}(PO_{4})_{6}F_{2}, chlorapatite (CA) Ca_{10}(PO_{4})_{6}Cl_{2} and hydroxyapatite (HA) Ca_{10}(PO_{4})_{6}(OH)_{2}. Bone apatites can be more accurately described...
as CO\textsubscript{3}-substituted apatites (CSA). Among the substituting ions in apatites that are reported in bone and tooth mineral and/or used in biomedical applications are F\textsuperscript{−}, Cl\textsuperscript{−}, B\textsuperscript{3+}, Cu\textsuperscript{2+}, Na\textsuperscript{+}, K\textsuperscript{+}, Fe\textsuperscript{3+}, Mg\textsuperscript{2+}, Mn\textsuperscript{2+}, Zn\textsuperscript{2+}, Si\textsuperscript{2+} or Sr\textsuperscript{2+} chloride, citrate, carbonate [9,11–17]. The choice of osteoblast cell lines to induce mineral formation is crucial since each cell line exhibits distinct characteristics of the extracellular matrix and mineralization.

Osteosarcoma-derived cells are commonly used as osteoblastic models [18]. Among the three osteosarcoma cell lines tested: MG-63, Saos-2 and U-2 OS, Saos-2 cells revealed the most mature osteoblastic labeling profile [18]. They are able to secrete MVs [19] and are good in vitro models of human osteoblast-like cell implant models [20]. Human bone marrow mesenchymal stromal cells (BMMSCs) are excellent model systems, however their scarcity, heterogeneity and limited lifespan restricted their use [20]. Undifferentiated human fetus hFOB cells possess similar markers as BMMSCs and are widely used as a model of normal osteoblastic differentiation [21]. In this report, we compared the formation of minerals by two selected cell models: osteosarcoma Saos-2 cells and human fetus hFOB 1.19 cells. Our aim was to determine the amount and composition of minerals, the activity of TNAP and the number of vesicles in these two different cell lines, to better understand mechanisms of mineralization during distinct stages of differentiation.

2. Materials and methods

2.1. Cell culture and treatment

Human fetus hFOB 1.19 SV40 large T antigen transfected osteoblasts (ATCC CRL-11372) were cultured in a 1:1 mixture of Ham’s F12 medium and Dulbecco’s modified Eagle’s medium with 2.5 mM \(\text{L-glutamine} \) (ATCC) supplemented with 100 U ml\(^{-1}\) penicillin, 100 U ml\(^{-1}\) streptomycin (Sigma), 0.3 mg/ml G418 (Sigma) and 10% FBS (v/v, Gibco). The cells were grown at 34 °C in atmosphere of 5% CO\textsubscript{2}.

Human osteosarcoma Saos-2 cells (ATCC HTB-85) were cultured in McCoy’s 5A medium with 1.5 mM L-glutamine (ATCC) supplemented with 100 U ml\(^{-1}\) penicillin, 100 U ml\(^{-1}\) streptomycin (Sigma) and 15% FBS (v/v, Gibco). The cells were grown at 37 °C in atmosphere of 5% CO\textsubscript{2}.

Cells were stimulated for mineralization by treatment with 50 \(\text{µg ml}^{-1}\) ascorbic acid (AA, Sigma) and 7.5 mM \(\beta\)-glycerophosphate (\(\beta\)-GP, Sigma) for 7 days [22].

2.2. Synthesis of apatites

2.2.1. Hydroxyapatite (HA)

Hydroxyapatite was obtained from commercially available powder \(\text{Ca}_{10} (\text{PO}_4)_{6} (\text{OH})_2\) (Bio-Gel HTP Gel, Bio-Rad Lab.). Ca to P ratio, calculated from the atomic numbers, is 1.67.

2.2.2. Fluorapatite (FA)

Fluorapatite was synthesized according to Yao et al., with modifications [23]. A mixture of 75 ml of 0.01 M phosphate buffer (\(\text{Na}_2\text{HPO}_4\) and \(\text{NaH}_2\text{PO}_4\) (Sigma) in a 1:1 ratio, pH 6.7) with 0.003 M NaF (Sigma) was prepared in a flask into which 25 ml of 0.05 M solution of \(\text{Ca}_2\text{H}_5\text{O}_2\)\(_2\) × \(\text{H}_2\text{O}\) (POCh) was added under constant mixing at 1000 rpm. Then, 20.21 ml of 0.2 M NH\textsubscript{4}Cl (Sigma), pH 9.0, earlier adjusted with 1.37 ml NH\textsubscript{4}OH (POCh), was slowly added. The reaction mixture was placed in an oven at 90 °C for 2 h with occasional stirring. The resulting precipitate was then left for 24 h at room temperature to mature. The chlorapatite crystals formed were filtered through filter paper (Whatman No. 3) and placed in glass beakers to dry at 80 °C for 24 h.

2.2.3. Chlorapatite (CA)

Chlorapatite was synthesized according to Kannan et al., with modifications [24]. A 52 ml solution of 1 M Ca(NO\textsubscript{3})\(_2\) × 4 \(\text{H}_2\text{O}\) (Sigma) was prepared in a flask, into which 30.64 ml of 0.6 M solution of \(\text{NH}_4\)\(_2\)\text{HPO}_4 (Sigma) was added under constant mixing at 1000 rpm.
دریافت فوری
متن کامل مقاله

امکان دانلود نسخه تمام متن مقالات انگلیسی
امکان دانلود نسخه ترجمه شده مقالات
پذیرش سفارش ترجمه تخصصی
امکان جستجو در آرشیو جامعی از صدها موضوع و هزاران مقاله
امکان دانلود رایگان ۲ صفحه اول هر مقاله
امکان پرداخت اینترنتی با کلیه کارت های عضو شتاب
دانلود فوری مقاله پس از پرداخت آنلاین
پشتیبانی کامل خرید با بهره مندی از سیستم هوشمند رهگیری سفارشات