Dissolution mechanisms of different types of bone mineral

Nazaret Domínguez-Gasca (1*), Cristina Benavides-Reyes (1), Estefanía Sánchez-Rodríguez (1), Alejandro B. Rodríguez-Navarro (1)

(1) Departamento de Mineralogía y Petrología, Universidad de Granada, 18002, Granada (España)
* corresponding author: nadoga@ugr.es

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INTRODUCTION

Bone is a composite material with a complex chemical composition and hierarchically structure that serves different functions such as structural support and supply of ions for homeostasis (Fratzl et al., 2004; Schmahl et al, 2016). It is mainly composed of an inorganic phase (nanocrystalline carbonated apatite) mineralizing an organic matrix (mainly type I collagen) and water. Bone is a living tissue that is under constant remodelation by bone cells (Bonucci, 2013). Given the heterogeneous composition and complex structure of bone, there is not a complete understanding of how bone mineral dissolution occurs which is needed to understand bone formation and resorption during the bone remodeling processes (Rey et al. 2009). It is also very important when studying alteration processes occurring in archeological bone during burying as well as during excavation and cleaning treatments (Schoeninger & Moore, 1992; Berna et al., 2004).
To better understand this process, we have studied in detail how bone mineral chemistry and structure change during demineralization using different analytical techniques such as 2D X-ray diffraction and infrared spectroscopy, optical and electron microscopy.

METHODOLOGY

Bone Samples

Tibiae samples (n =16) from White Leghorn laying hens (62 weeks old) were selected for this study. One cm thick slices were cut from each tibia at mid-diaphysis to prepare longitudinal pieces of cortical bone (about 10 x 5 x 0.5 mm in size) and to extract the medullary bone (25 mg) from the marrow cavity.

Demineralization experiments

Longitudinal pieces of cortical bone were immersed in 1mL of 1% HCl and 5 % HCl acid solution under stirring for different times: 5, 30, 60, 300, 1440 min. Medullary bone samples were kept also in 1% or 5% HCl acid solution for 5, 10, 30, 60 min. After the treatments, samples were rinsed twice with 20 mL of distilled water to remove any residual of hydrochloric acid, and dried at room temperature for 24 hours.

Optical microscopy and Electron microscopy

Serial histological sections, 5 μm thick, were stained with toluidine blue and with a mixture of picrosirius red - fast green to examine the medullary bone with fluorescence microscopy (Leica DMRB). Scanning electron microscopy (SEM) observation was carried out on polished cross-sections of the tibiae mid-diaphyses using a variable pressure SEM (LEO 1430-VP) and ultra thin-sections of untreated cortical and medullary bone were observed using a Carl Zeiss LIBRA 120 PLUS TEM (Germany).

Infrared spectrometry

Homogenized samples in powder form were analyzed using a FTIR spectrometer (model 6200, JASCO Analytical Instruments Japan) to determined different compositional parameters and follow the changes in chemical composition of bone tissues (cortical and medullary bone) during demineralization.

Two-dimensional (2D) X-ray diffraction

Crystallinity of bone mineral was determined in longitudinal sections of tibiae cortical bone and were analyzed with a single crystal diffractometer equipped (D8 Venture, Bruker, Germany) with a PHOTON area detector, Mo radiation and a beam size of 0.2 mm in diameter using Mo radiation.

RESULTS

The cross-section tibiae at mid-diaphyses shows the two structurally distinct types of bone (cortical and medullary bone; Fig 1A). The outermost part of the bone made of

compact cortical bone is constituted by cylindrical bone structural units(osteons) which grow by the accretion of lamellar bone. However, the medullary bone, located in the marrow cavity, is made up of disperse and small bone trabeculae. The different chemical composition of cortical and medullary bone causes them to stain differently in histological sections prepared with picrosirius red and fast green (Fig 1B). The cortical bone is stained with an intense green, and the medullary bone is stained in red.

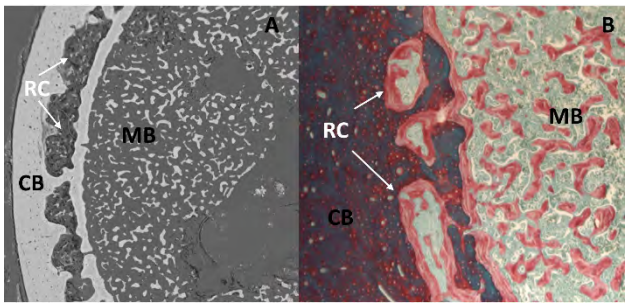

Fig 1*. A) Variable pressure electronic microscopy (VPSEM) image of tibiae cross-section from laying hens. CB: cortical bone; MB: medullary bone; RC: resorption center. Scale bars: 200 □ m. B) Transverse section of tibiae stained with red picrosirius and fast green to visualize collagen fibers.*

Compositional parameters determined by FTIR shows that during bone demineralization, specific mineral components are selectively and progressively removed. The degree of mineralization (measured as PO4/Amide I peak area ratio) of cortical bone decreases gradually with the time of exposure to the acid solution, being the decrease more rapid as the concentration of the acid increases (1 % HCl versus 5%HCl solution). At both acid concentrations, the demineralization of cortical bone is completed after about 5 hours and no phosphate is detected after that time. During acid demineralization, the carbonate and phosphate components are lost simultaneously as both components are associated in the bone mineral. However, the total amount of carbonate in the bone mineral compared to carbonate substituted (mainly type B) in bone mineral decreases gradually with the time. This data indicates that labile carbonate from the mineral hydrated layer is selectively and more rapidly removed during demineralization than carbonate substituted in the apatitic mineral core (mainly type B carbonate).

In medullary bone, the demineralization of medullary bone decreased very rapidly with time reaching zero in less than 30 min when is exposed to 1 % HCl solution and in 5 minutes or less when is exposed to 5 % HCl solution. In this tissue, most of the carbonate is labile being the CO3_1415 /CO3_870 ratio greater than in cortical bone

The analysis of 2D X-ray diffraction patterns of bone provides detailed information on the bone mineral crystallinity and its structural organization. Cortical bone as apatite crystals are preferentially oriented with their c-axis parallel to the long axis of bone however medullary bone as apatite crystals are randomly oriented. During acid induced demineralization the FWHM values of 002 reflections gradually decrease with time. The angular spread decreases at the beginning of the demineralization process (first 5 minutes) and remains nearly constant later on. These results indicate that the less crystalline, and more disordered mineral fraction (i.e., smaller randomly oriented apatite crystals), is preferentially removed in the cortical bone mineral during the demineralization process.

CONCLUSIONS

In conclusion, bone mineral dissolution is highly complex process as it is dependent on multiple factors such as bone mineral characteristics (crystal size, amount and type of impurities, crystallinity) which determine its stability, reactivity and solubility. Additionally, bone organic matrix composition and its structural relationship with the mineral also strongly influences bone mineral solubility in different bone types. Furthermore, bone is a highly heterogeneous material which dissolves incongruently.

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