Highlights and Breakthroughs: Thermodynamic Approach Provides Insights into the Ageing Process of Biological Apatite

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Running title: Thermodynamic approach to bioapatite

Abstract

In a recent of issue of this journal, Rollin-Martinet *et al.* (American Mineralogist, 98, 2037-2045) take a thermodynamic, in contrast to a medical-biological, approach to the maturation process of biological apatite. They do so by focusing on changes in the HPO_4^{2-} concentration in biomimetic apatite over time. In this first-of-its-kind analysis, they conclude that the increase in stability of bone mineral over time ultimately demands that bone be remodeled (i.e., replaced by new bone) in order for the mineral to retain its biologically important functional properties.

Key words: bone, bioapatite, maturation, remodeling, thermodynamics

The fact that the apatitic biomineral phases of bone and tooth enamel reveal signs of their ageing is no surprise to either medical researchers or mineralogists (Eanes and Meyer 1977). Medical researchers attribute such changes to biologically controlled processes. Mineralogists and geochemists, however, look to possible thermodynamic driving forces to account for changes over time in mineral crystallites that continue to reside in an aqueous (body) fluid that is very similar to that from which they initially precipitated. In a recent issue of the journal, Sabrina Rollin-Martinet and colleagues (Rollin-Martinet et al. 2013) investigate the chemical evolution of apatite in bone and in tooth enamel by means of thermodynamic analysis, based on solution calorimetry and chemical-structural monitoring of synthetic analogs to bioapatite.

The authors specifically address the thermodynamic basis underlying (1) the maturation of bioapatite, which occurs in all known types of apatite biomineralization, and (2) remodeling (also known as turnover), which occurs in the bones of many vertebrates, but not in short-lived ones such as rodents. Because bone remodeling is such an important factor in the health of human bones, the authors critically evaluate the thermodynamic necessity of this biologically-controlled process, in which one set of specialized cells dissolves a selected volume of bone (both mineral and collagen) and another set replaces it with new bone material. Tooth enamel, in contrast, undergoes maturation but not remodeling (Glimcher 2006).

Rollin-Martinet *et al's* (2013) thermodynamic analysis of bioapatite is the first of its kind, in that other researchers have relied on the data for stoichiometric, "coarse-grained" hydroxylapatite [$Ca_{10}(PO_4)_6(OH)_2$] in lieu of the chemically more complex, non-stoichiometric, nanocrystalline bioapatite that is better modeled as $Ca_{10-x-y}(PO_4)_{6-x-y}(CO_3)_x(HPO_4)_y(OH)_{2-x-y}$ (Cazalbou et al. 2004). The major simplification they allow themselves is to treat only the HPO_4^{2-} and not the CO_3^{2-} substituent in their current study, making sure in their synthetic materials that CO_2 was excluded from the growth environment.

There are multiple types (by composition, crystallite size, degree of crystallinity) of nanocrystalline calcium phosphate biomineralization, typically involving nanocomposites of mineral and organic molecules, such as collagen. The properties of these nanocomposites are to some degree optimized for specific biological functions (Daculsi et al. 1997): Enamel protects the tooth from chemical and mechanical attack, making low solubility and elevated hardness desirable. In contrast, bone mineral acts as an ion reservoir and must be moderately reactive and soluble in order to respond as rapidly as needed to release important ions.

In their analysis and interpretation, Rollin-Martinet *et al.* account for the extremely large surface-area-to-volume ratio that is afforded by the nanocrystalline size and platelet shape of bioapatite. They use FTIR spectroscopy to distinguish between structurally incorporated (i.e., apatitic) and surface-bound (i.e., non-apatitic) ions. The latter ions are relatively abundant in nanocrystals and important to their chemical reactivity, e.g., the crystals' chemical affinity for associated organic molecules and their ability to undergo ion exchange. Previous studies have shown that ageing leads to an increase in size, a decrease in surface reactivity and in HPO_4^{2-} concentration, but an increase in CO_3^{2-} concentration within bioapatite crystals (Glimcher 2006; Boskey and Coleman 2010).

Recognizing that the rate of change during the maturation process drops off strongly in the earliest stages (first three days), the authors analyzed samples that resided in their precipitation solutions from 20 minutes to three weeks. X-ray diffractometry (XRD) recorded the expected increase in crystallinity of the synthetic apatite over time. Changes in XRD peak widths permitted the inference of crystallite growth from 12 to 27 nm in longest dimension during this time period. While HPO_4^{2-} content decreased with maturation, Ca^{2+} and OH^- contents increased, as did the Ca/P atomic ratio. In other words, the apatite began to approach the stoichiometry of hydroxylapatite.

Evaluation of the enthalpies of formation of the synthesized apatite samples showed increasingly negative values as maturation progressed. Such trends parallel those of the above compositional changes in the apatite. Because the entropy of formation is exceedingly small, the Gibbs free energy essentially follows the enthalpy trends, i.e., becoming increasingly

negative during maturation. These assessments for the first time allowed quantitative tracking between the energy of formation of biomimetic apatites and the actual ion concentrations in the samples. The bottom line is that HPO_4^{2-}-rich bioapatites and their synthetic analogs become more stable over time. For tooth enamel, the increased stability is an advantage to its protection capabilities. For bone, increased stability means lower solubility and chemical reactivity, thereby degrading the bone mineral's ability to stabilize ion concentration ratios in the body fluid (i.e., homeostasis) through controlled dissolution. Thus, bone must be remodeled on a regular basis in order to maintain the biologically necessary properties of the mineral.

An additional biological advantage to the remodeling of bone is that the new bioapatite crystallites are smaller than the mature ones, which is important to the spatial accommodation of the mineral within the collagen matrix/framework of the bone (Alexander et al. 2012). The functional necessity of bone remodeling is thus made apparent by both thermodynamic and geometric analysis. How the thermodynamic inconvenience of bioapatite maturation translates into a biological inducement for bone remodeling, however, is yet another story. It is to be hoped that in the future the authors will address the thermodynamic effects of the highly significant carbonate constituent in bone and tooth apatite. The current study illustrates the strength of combining one group's long history of laboratory-based analysis of biological and synthetic apatite with another's careful calorimetry and thermodynamic analysis.

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