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Comparing bioapatite carbonate pre-treatments for isotopic measurements: Part 1—Impact on structure and chemical composition

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ABSTRACT

A large variety of bioapatite pre-treatments are found in the literature for a wide range of applications going from mineralogy to diagenesis. Additionally, stable carbon and oxygen isotopes in bioapatite carbonates are often measured after pre-treatment to study ancient diets and reconstruct past environments. It is common to pre-treat the samples to remove contributions from competing sources associated to organic material and secondary carbonates. Several chemical methods are described in the literature to pre-treat archaeological and fossil bioapatites (bone and teeth) for isotopic measurements with no consistency on their efficiency. Infrared and elemental analyses are combined in this study to highlight the effects of the different pre-treatments on the structure and chemistry of bioapatite. The different pre-treatments can variably affect the organic and carbonate contents of bioapatites, with some, such as sodium hypochlorite, apparently adding carbonate to the system. This may lead to a change in the biological isotopic signature of bioapatite carbonate, with effects on the correct interpretation of the results. According to the findings of our study, hydrazine hydrate seems to be the best chemical to remove organic matter without affecting the carbonate content of bioapatites, while the use of acetate buffered acetic acid solutions is recommended for removing adsorbed carbonates, when necessary. Sodium hypochlorite, instead, induces the adsorption of exogenous carbonates, while hydrogen peroxide does not remove organic matter efficiently even at high temperatures. These two chemicals should not be used to remove organic matter, especially from bone and dentine.

1. Introduction

When studying the mineral fraction of bone and teeth (bioapatite), it is often necessary to remove other skeletal components such as organic matter and/or contamination from secondary carbonates. To carry out these separations, several chemical protocols have been employed in the literature. Research in bioapatite includes investigation on its mineralogical structure (e.g. Tomazic et al., 1993; Ichinohe et al., 2010), and diagenetic or taphonomic (post mortem) chemical and structural modifications (e.g. Nielsen-Marsh and Hedges, 1999).

Another important suite of studies are carried out on the bioapatite isotope composition. The measurement of isotope ratios in mammal skeletons of fossil or archaeological material is used to investigate past lives and ecology of humans and animals. When measuring oxygen and carbon isotope compositions of the bioapatite carbonate compartment in these materials, the aim is to analyse the biological signature in this mineral fraction, which has to be separated from the coexisting organic material (approximately 25–30% in bone, 20% in dentine, and 0.5–2% in enamel—Stack, 1955; Driessens and Verbeeck, 1990). This separation is necessary because stable isotopes are differently fractionated in the organic and inorganic fractions of skeletal tissues. In addition, part of the mineral phase can be contaminated by exogenous (secondary) carbonate and/or diagenetic apatite. Both organic matter and exogenous mineral phases should be removed from the samples in order to grant the measurement of the pristine isotope compositions of bioapatite carbonate.

Several pre-treatment methods have been proposed over the last few decades to remove organic matter and exogenous minerals. Sodium hypochlorite (NaClO) and hydrogen peroxide (H_2O_2) are the most commonly used reagents to eliminate organic matter from bioapatites (e.g. Koch et al., 1997), while acetic acid (CH_3COOH) is often employed to remove adsorbed and diagenetic carbonates that are believed to be more soluble than unaltered biological apatite (e.g. Garvie-Lok et al., 2004). However, it has been observed that these treatments have an impact on the measured carbon and oxygen isotope ratios of bioapatite carbonates and it is not clear if these variations are due to the removal of organic matter and secondary minerals and/or to other factors (e.g. Koch et al., 1997). Already in 1997, Koch and colleagues understood that '*The key to obtaining consistent results will be extreme consistency*

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in concentration of solutions used, in the proportion of solution to sample, and in the time for each treatment step'. Nevertheless, in recent years, it is still possible to find a plethora of different sample pre-treatments used for the isotopic study of bioapatite carbonate (e.g. Forbes et al., 2010; Brookman and Ambrose, 2012; Loftus and Sealy, 2012); some also advocate that, because of these variations in isotope values, in some cases it is best not to carry out any pre-treatment at all (Zazzo et al., 2006; Pellegrini et al., 2011). A recent study comparing the effect of H_2O_2 and NaClO on the carbon and oxygen isotope composition of bioapatite carbonate recommended the use of H_2O_2 at room temperature (Crowley and Wheatley, 2014). The main aim of this paper is to compare the effects and efficiency of the most recurrent pre-treatment methods on a variety of modern and fossil test samples.

1.1. Structure and chemical composition of bioapatites

Bones and teeth are composed of three main constituents: water, organic matter, and minerals. Here, the focus is on the inorganic mineral fraction, a highly substituted hexagonal calcium phosphate apatite commonly called bioapatite (LeGeros, 1991). Calcium phosphate apatite is composed of four essential elements: calcium, phosphorous, oxygen and an appropriate channel-filling ions such as chlorides, fluorides or hydroxyl groups: $Ca_{10}(PO_4)_6(Cl/F/OH)_2$. The composition of apatite is characteristically much more flexible than that of most minerals: it accommodates chemical substitution relatively easily. The most symmetric apatite is fluoroapatite as fluoride (F^-) is smaller than both chloride and hydroxyl groups. When hydroxyl groups occupy this space (hydroxyapatite—HAP—$Ca_{10}(PO_4)_6(OH)_2$), distortions are created because of their size, non-spherical form and charge arrangement (Wopenka and Pasteris, 2005).

Bioapatite is similar, but not identical, to hydroxyapatite in which non-stoichiometric substitutions are possible (Lee-Thorp, 2002). Phosphate groups (PO_4^{3-}) are partially replaced by carbonates (CO_3^{2-}) and most hydroxyl groups (OH^-), if not all in bone, are also replaced by carbonates (Pasteris et al., 2004; Skinner, 2005; Wopenka and Pasteris, 2005). Carbonates that replace hydroxyl groups and phosphates are known as type A and B carbonates respectively (LeGeros et al., 1969; LeGeros, 1991). Carbonate not only substitutes for phosphate and hydroxyl groups within the crystal structure of bioapatite ('structural carbonate') but can also be adsorbed onto the surface of crystals and hydration layers ('adsorbed carbonate') (LeGeros, 1981). Water is often found adsorbed on the bone crystallites (hydration layers) but can also be found within the structure of bone apatite, probably replacing the channel filling ions (Yoder et al., 2012). The composition of bioapatite can be approximated with the following formula where [] represents potential vacancies in the structure (LeGeros et al., 1986; Skinner, 2005; Wopenka and Pasteris, 2005; Yoder et al., 2012):

$$(Ca, Na, Mg, Ba, Fe, Sr, Zn, [\ ])_{10}(PO_4, HPO_4, CO_3)_6(OH, F, Cl, CO_3, O, H_2O, [\ ])_2$$

Since carbonate has a different geometry and charge to phosphate and as it is larger than hydroxyl, its presence in the crystal structure of bioapatite leads to lattice distortions of the hexagonal structure, and to smaller crystal size compared to HAP (LeGeros, 1991, 2008). The crystallinity of apatite—indication of lack of defects (or distortions and strains) and crystal sizes (LeGeros, 1991)—is therefore lower in bioapatite than HAP. In bone and dentine apatite, because of the small crystal size, the area/mass ratio is very high, resulting in a higher reactivity and solubility compared to tooth enamel and HAP (LeGeros, 1991). Bone and dentine apatite are therefore more susceptible to post-mortem alterations or diagenesis. Tooth enamel has fewer substitutions, less distortions and larger crystals than bone and dentine apatite (LeGeros, 1991) rendering it more stable to post-mortem alterations (Lee-Thorp and Sponheimer, 2003).

1.2. Current pre-treatments to remove organic matter

In bone and dentine, collagen is the major organic component, while in enamel the small amount of organic matter is partially made of keratin (Stack, 1955). The usual way of removing organic matter is by oxidation. Two oxidising agents are commonly employed in bone and teeth for this purpose: sodium hypochlorite (NaClO) and hydrogen peroxide (H_2O_2). Different concentrations are found in the literature for both reagents: from 1 to 5% for NaClO and from 10 to 30% for H_2O_2. While all treatments involving NaClO are carried out at room temperature, some users of H_2O_2 perform their treatments at higher temperatures (e.g. O'Neil et al., 1994). Both are oxidising agents; NaClO is a base, with a pH going up to 11 at 5%, while H_2O_2 is an acid, with a pH going down to 5 at 30%. Another oxidising agent used in the pre-treatment of calcined bone for radiocarbon dating is sodium chlorite (1.5% $NaClO_2$ at pH 3—Brock et al., 2010). Hydrazine hydrate (95% $N_2H_4{\cdot}H_2O$) is another reagent that has seldom been used for organic removal before isotopic measurements of bioapatite carbonates (Termine et al., 1973; Nielsen-Marsh and Hedges, 1999). $N_2H_4{\cdot}H_2O$ is also a base with a pH of circa 11 but, differently from NaClO, it is a reducing agent.

A final way to remove organic matter is by heating, though this has a significant impact on the composition and structure of bioapatites (Lebon et al., 2010; Grimes and Pellegrini, 2013; Snoeck et al., 2014).

1.3. Current pre-treatments to remove exogenous minerals

The potential adsorbed or secondary (diagenetic) carbonates also need to be eliminated from the samples. Acetic acid (CH_3COOH) is the most commonly used reagent to remove the exogenous carbonates from bioapatite samples. The effects of acetic acid on the structure and isotope composition of bioapatites have already been discussed in previous studies. Most often, concentrations between 0.1 and 1 M have been used, and acetate buffered solutions as well (Lee-Thorp and van der Merwe, 1991; Koch et al., 1997; Garvie-Lok et al., 2004; Yoder and Bartelink, 2010; Crowley and Wheatley, 2014). Duration of treatment is very variable, from 10 min up to 48 h. However it has been shown that prolonged treatments with acetic acid have a drastic effect on the structure of bioapatites, which tends to be transformed into brushite (Lee-Thorp and van der Merwe, 1991).

1.4. Aims of this study

This study stems from the need for a better understanding of how pre-treatments actually affect the structure and chemical composition of bioapatites. Additionally, there is a need for more consistency in pre-treatment methods used in the isotope analyses of bioapatite carbonates. Several studies have already discussed the effects of acetic acid on the isotope composition of bioapatite carbonates (e.g. Lee-Thorp and van der Merwe, 1991; Garvie-Lok et al., 2004, Yoder and Bartelink, 2010) but only very few have assessed the effects of the several organic matter removal pre-treatments employed in palaeoclimate and palaeodietary studies (e.g. Koch et al., 1997; Zazzo et al., 2006; Grimes and Pellegrini, 2013; Crowley and Wheatley, 2014).

While many researchers have investigated the impact of different pre-treatments on the isotope composition of bone apatite, few actually checked what effects these pre-treatments had on the chemical composition and structure of bioapatites. In other words, do the pre-treatments used to remove organic matter from bioapatite actually remove it efficiently? And are adsorbed/diagenetic carbonates the only mineral fractions removed during acetic acid pre-treatments? Here, elemental and infrared analyses are combined to understand the effects of different pre-treatments on the chemical composition but also on the structure of the samples. A range of archaeological and modern samples, including bone apatite, tooth enamel, and tooth dentine, are included in this study.