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Is X-ray diffraction able to distinguish between animal and human bones?

Giampaolo Piga [a,b,*], Giuliana Solinas [c], T.J.U. Thompson [d], Antonio Brunetti [e], Assumpció Malgosa [a], Stefano Enzo [b]

[a] GROB (Grup de Recerca en OsteoBiografia), Unitat d'Antropologia Biologica, Department de Biologia Animal, Biologia Vegetal i Ecologia, Universitat Autonoma de Barcelona, Edifici C, 08193 Bellaterra, Barcelona, Spain
[b] Dipartimento di Chimica e Farmacia, Università di Sassari, via Vienna 2, I-07100 Sassari, Italy
[c] Department of Biomedical Sciences, Laboratory of Epidemiology and Biostatistics, University of Sassari, via Padre Manzella 4, I-07100 Sassari, Italy
[d] School of Science & Engineering, Teesside University, Borough Road, Middlesbrough TS1 3BA, UK
[e] Struttura Dipartimentale di Matematica e Fisica, Università di Sassari, via Vienna n. 2, I-07100 Sassari, Italy

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A B S T R A C T

The possibility of determining the human or animal origin of bones from the lattice parameters of their inorganic bioapatite phase, when subjected to a high temperature treatment using the powder X-ray diffraction (XRD) technique, has been explored on a wide number of specimens. Forty-two animal bones were treated in a furnace at 1100 °C for 36 min and compared to 53 cremated human bones from a range of ancient necropolises. The X-ray diffraction patterns of bioapatite were simulated using both monoclinic P21/b and hexagonal P63/m structures to verify any occurrence of phase transformation and any difference in the lattice parameters due to the model. It was determined that the differences between the *a*-axis and *c*-axis of the monoclinic and hexagonal lattice were unimportant. Some outlying values were revealed to be caused by the presence of chlorine ions diffused into the apatite structure increasing its average unit cell values. Nevertheless, our results clearly show that in terms of lattice parameters the variability of human specimens are completely overlapped by the non-human variability making the use of XRD in order to distinguish animal from human bones questionable.

1. Introduction

The separation of animal from human bone is an important component of any archaeological or forensic osteological and histological analysis (Cattaneo et al., 1999; Cuijpers, 2006; McKinley, 1994; Whyte, 2001). It can be important for a range of reasons, from determining the minimum number of individuals present, to understanding funerary behavior, to comprehending human–faunal relations. This is also true of burned skeletal material, but this work is greatly complicated by the range of heat-induced changes that bone undergoes when burned (Thompson, 2005). Thus studies which focus on the separation of different species of bone, especially if fragmented, are extremely valuable. With this in mind, Beckett et al. (2011) reported in a recent paper the possibility of determining the human rather than animal origin of bone from the lattice parameters of the inorganic bioapatite phase from the diffraction patterns of bones subjected to a high temperature heating treatment. Actually, the structural properties of a substance are inspected by diffraction in terms of symmetry operations compatible with three-dimensional periodicity of the crystals, i.e., specifying one of the 230 possible space groups (see: International Tables for X-ray Crystallography, 1965–68), complemented with the geometry and dimensions of the unit cell of the lattice (so-called lattice parameters) as well as its atomic content and arrangement. For the case of bioapatite crystals found in bones, a space group $P6_3/m$ is generally attributed with a hexagonal unit cell where two lattice parameters *a*- and *c*-axis respectively, need to be determined. According to Beckett et al. (2011), the plot of *a*- vs *c*-axis data points from human being occurs in a typical and distinct area with respect to animals.

The determination of lattice parameters depends upon the precision of locating the peak profiles in XRD diagrams (Masciocchi and Artioli, 1996), but in bioapatite this is difficult to do. This is because of large peak broadening resulting from the small crystallite size of the phase combined with the high amount of lattice strain (Danilchenko et al., 2002). To alleviate this problem, Beckett et al. (2011) have suggested that lattice parameter determination be performed on highly crystallized single phase materials following

* Corresponding author. GROB (Grup de Recerca en OsteoBiografia). Unitat d'Antropologia Biologica, Department de Biologia Animal, Biologia Vegetal i Ecologia, Universitat Autonoma de Barcelona, Edifici C, 08193 Bellaterra, Barcelona, Spain.
E-mail address: kemiomara@yahoo.it (G. Piga).

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thermal treatment of the bone. However this in turn creates a potential problem in identifying the most appropriate heating temperature for differentiating faunal from human bone.

Another potential issue with the approach in Beckett et al. (2011) stems from the fact that their analysis is limited to a sample of just 8 human specimens vs 65 non-human samples from 12 different species. This may be due to the difficulties in acquiring modern bone for such research, but nevertheless the large availability of human bones from the archaeological context offers considerable scope for the continued investigation of this area (Piga et al., 2007). Thus we have critically investigated the diffraction patterns of a wide variety of bones originating from various contexts routinely met in the course of our archaeological and anthropological investigations (Piga et al., 2010a, 2010b).

Unfortunately the factors regulating the chemistry of bones are still not completely known. Apatites have the general formula, $Ca_5(PO_4)_3X$ or $Ca_{10}(PO_4)_6X_2$ where X is typically F (fluorapatite), OH (hydroxylapatite), or Cl (chlorapatite) in case of natural minerals (Elliott et al., 2002). Typically the mineral of bone and teeth is an impure form of OHA where the major variations in composition focus on a variable Ca/P mol ratio (1.6–1.7, OHAp is 1.66), and a few percent CO_3^{2-} and water. In fact, the apatite lattice is very tolerant to substitutions, vacancies and solid solutions; for example, X in the general chemical formula above can be replaced by $½CO_3^{2-}$ or $½O^{2-}$; Ca^{2+} by Sr^{2+}, Ba^{2+}, Pb^{2+}, Na^{+} or vacancies; and PO_4^{3-} by HPO_4^{2-}, AsO_4^{3-}, VO_4^{3-}, SiO_4^{4-} or CO_3^{2-}. It is the degree of such substitutions that can affect the average lattice parameter values and introduce some voids or strain (Aellach et al., 2010), and these may also be responsible for the unique mechanical properties of bone. Other factors affecting the lattice parameter are the presence of organic materials of biogenic origin, and extra phases (Elliot, 1994).

Wopenka and Pasteris (2005) have recently discussed the oversimplifications involved when using the hydroxylapatite inorganic phase as a model of bones, especially in view of the types of ionic substitutions that can occur in the apatite lattice which may then change the mineral characteristics of the bone material. Instead, Wopenka and Pasteris (2005) locate natural bioapatite inside a hyper-phase diagram with end-members of apatite minerals such as hydroxylapatite, fluorapatite, A-type carbonated apatite, B-type carbonated fluorapatite (formerly known as francolite), and B-type carbonated hydroxylapatite (formerly known as dahllite).

Of course, *post-mortem* taphonomic and diagenetic changes are expected to add further complexity to the structure and microstructure of bones, not only due to new ionic substitutions but also in terms of new biogenic or authigenic phases that form during the conservation, storage and degradation processes of bone (Shinomiya et al., 1998; Piga et al., 2009a, 2011).

The paper by Beckett et al. (2011) has employed a simplified approach for lattice parameter determination starting from the peak positions which are calculated by the automatic location of the maxima of diffraction patterns (which may not be completely satisfactory). In our work care has been exercised in order to measure the lattice parameters of the bioapatite phase with the best practices ensuring precision and accuracy. The Rietveld method (Rietveld, 1967; Young, 1993) appears to be the most orthodox approach for this purpose (Peterson, 2005) and indeed is now standard practice in materials science (although its use has appeared only sporadically in the archaeological and forensic fields). Another important point concerns the most suitable space group for describing the bioapatite structure when using powder XRD. While the most popular space group to represent the structure of bioapatite is $P6_3/m$, a more suitable alternative appears to be a monoclinic description using the $P2_1/b$ space group. This is due to the fact that OH− is non-spherical and therefore reduces possible crystalline symmetry (Elliott et al., 1973; Wopenka and Pasteris, 2005). Moreover, we must also bear in mind that it was recently reported that a monoclinic-to-hexagonal order/disorder transformation occurs at 220 °C for synthetic apatite (Yashima et al., 2011).

In this work, first we address the problem of whether the monoclinic $P2_1/b$ vs hexagonal $P6_3/m$ space group can make a substantial difference in terms of lattice parameter values for the bioapatite of bones. We then evaluate the most evident structural changes involved after high-temperature treatment. Finally we discuss the lattice parameter values of heat-treated animal and human bone samples from various Spanish and Italian necropolises.

2. Experimental methodology

2.1. Examined specimens

The forty-two animal bone specimens were kindly made available from: the Institut Català de Paleontologia (Sabadell-Barcelona, Spain), the School of Science & Engineering, Teesside University (UK), and the Department of Animal Biology, University of Sassari (Italy). Our collection consists of 25 species, distributed as it follows: mammoth (3), monkey (3), camel (1), deer (2), rhino (1), horse (2), ox (1), pig (1), ruminant (2), sheep (1), goat (2), rodent (1), lagomorph (2), cat (1), lion (1), dog (1), fox (1), crocodile (1), turtle (2), bird (6), whale (1), dolphin (3), tuna (1), swordfish (1), shark (1). The specimens date from the present time back to 900,000 years ago.

Further, the fifty-three human bones were kindly made available from: the Universitat Autonoma de Barcelona (Spain), and the Department of History, University of Sassari (Italy). These bones originate from: the Necropolis of *Aguilar de Montuenga* (Soria, Spain), the Necropolis of *Son Real* and *S'Illot des Porros* (Mallorca, Spain) (Piga et al., 2010b), the Necropolis of Sebès (Tarragona, Spain) (Belarte and Noguera, 2008), the Necropolis of *Mas d'en Boixos* (Pacs del Penedès, Alt Penedès, Spain) and the Necropolis of *Monte Sirai* (Carbonia, Italy) (Guirguis, 2010). Synthetic powder hydroxylapatite was synthesized by Aldrich Chemistry®.

2.2. Thermal treatment

In the present study we have selected historical human bones burned at temperatures above 1000 °C. This is based on our previous laboratory calibrations (Piga et al., 2008, 2009b). The animal bones were subjected to a heat treatment at 1100 °C for 36 min in a furnace, in order to sharpen the peak profiles to be used for determination of the lattice parameters.

2.3. Diffraction data collection and analysis

Exactly 0.5 g of each bone was ground in an agate jar for 1-min using a SPEX mixer-mill model 8000. Our sample holder for XRD analysis has a circular cavity of 25 mm in diameter and 3 mm in depth, and can hold 420 mg of pressed powder bone.

The Bruker D8 instrument was employed in the Bragg-Brentano geometry using fixed wavelength CuKα radiation and a graphite monochromator in the diffracted beam. The patterns were collected with a scintillation detector in the 2θ angular range from 9 to 140°, with a step-size of 0.05°; the counts at each data point being accumulated for 40 s in order to ensure accurate statistics for the intensity data and to reduce the uncertainty associated with the determination of lattice parameters. The X-ray generator worked at a power of 40 kV and 40 mA and the resolution of the instruments (0.5° divergent and 0.1 mm antiscatter slits) was determined using