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Adsorption of benzoxaboroles on hydroxyapatite phases

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ABSTRACT

Benzoxaboroles are a family of molecules that are finding an increasing number of applications in the biomedical field, particularly as a "privileged scaffold" for the design of new drugs. Here, for the first time, we determine the interaction of these molecules with hydroxyapatites, in view of establishing (i) how benzoxaborole drugs may adsorb onto biological apatites, as this could impact on their bioavailability, and (ii) how apatite-based materials can be used for their formulation. Studies on the adsorption of the benzoxaborole motif ($C_7H_7BO_2$, referred to as BBzx) on two different apatite phases were thus performed, using a ceramic hydroxyapatite (HA_{ceram}) and a nanocrystalline hydroxyapatite (HA_{nano}), the latter having a structure and composition more similar to the one found in bone mineral. In both cases, the grafting kinetics and mechanism were studied, and demonstration of the surface attachment of the benzoxaborole under the form of a tetrahedral benzoxaborolate anion was established using ^{11}B solid state NMR (including ^{11}B-^{31}P correlation experiments). Irrespective of the apatite used, the grafting density of the benzoxaborolates was found to be low, and more generally, these anions demonstrated a poor affinity for apatite surfaces, notably in comparison with other anions commonly found in biological media, such as carboxylates and (organo)phosphates. The study was then extended to the adsorption of a molecule with antimicrobial and antifungal properties (3-piperazine-bis(benzoxaborole)), showing, on a more general perspective, how hydroxyapatites can be used for the development of novel formulations of benzoxaborole drugs.

Statement of Significance

Benzoxaboroles are an emerging family of molecules which have attracted much attention in the biomedical field, notably for the design of new drugs. However, the way in which these molecules, once introduced in the body, may interact with bone mineral is still unknown, and the possibility of associating benzoxaboroles to calcium phosphates for drug-formulation purposes has not been looked into. Here, we describe the first study of the adsorption of benzoxaboroles on hydroxyapatite, which is the main mineral phase present in bone. We describe the mode of grafting of benzoxaboroles on this material, and show that they only weakly bind to its surface, especially in comparison to other ionic species commonly found in physiological media, such as phosphates and carboxylates. This demonstrates that administered benzoxaborole drugs are unlikely to remain adsorbed on hydroxyapatite surfaces for long periods of time, which means that their biodistribution will not be affected by such phenomena. Moreover, this work shows that the formulation of benzoxaborole drugs by association to calcium phosphates like hydroxyapatite will lead to a rapid release of the molecules.

1. Introduction

The unique reactivity of the benzoxaborole group [1–3] as well as its lack of toxicity [3–5] have recently attracted much attention for the development of new drugs (Fig. 1). Indeed, over the past ten years, a series of therapeutic molecules containing the benzoxaborole function have been synthesized, with antibacterial, antiviral, anticancer, anti-parasitic or anti-inflammatory activities [1,6]. Among these, tavaborole is worth mentioning (AN2690 – Fig. 1b), as it was recently commercialized by Anacor Pharmaceuticals for the topical treatment of onychomycosis [1,6]. Another important

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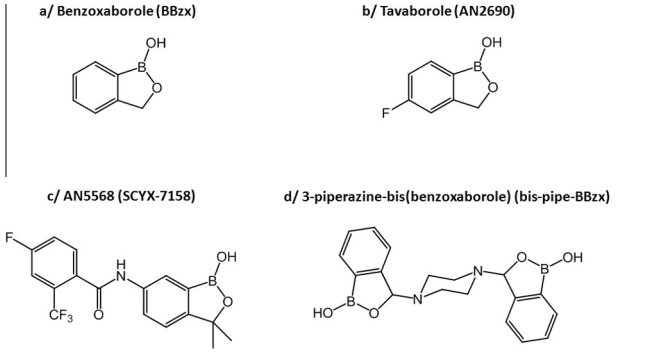

Fig. 1. Representation of the benzoxaborole motif BBzx (a), and of benzoxaborole molecules with therapeutic properties against fungi (b and d) or trypanosomes (c) [6,10].

molecule under investigation is AN5568 (also referred to as SCYX-7158 – Fig. 1c), an oral drug candidate against human African trypanosomiasis (sleeping sickness) [1,6]. The ability of the benzoxaborole group to bind to diols at physiological pH has also been exploited for other biomedical applications, like the internalization of enzymes in cells [7], the separation of sugars [8], or the rapid enrichment of glycosylated proteins [9].

While many studies have looked into the reactivity and biological activity of benzoxaboroles in solution [1,10], little is known as to how these molecules interact or react with common biomaterials, such as those used for the formulation of drugs or the elaboration of implants. It is only recently that our group started to study the association of simple benzoxaboroles (including AN2690) with inorganic biomaterials like layered double hydroxides (LDH) and with organic polymers like poly-L-lactic acid (PLLA) [11,12]. In both cases, the benzoxaboroles were incorporated into the biomaterials, their local environment was analyzed using techniques like solid state NMR, and their release in simulated physiological media was studied. We found that performing such investigations from a fundamental perspective was necessary because it allowed evaluating the intrinsic reactivity and stability of the benzoxaborole function with respect to these biomaterials, and drawing conclusions which are of importance for the development of benzoxaborole drug formulations.

One unexplored aspect is the reactivity of benzoxaboroles with respect to natural biominerals like hydroxyapatite. These studies are necessary not only because hydroxyapatites, and more generally calcium phosphates, can be used for the formulation of drugs [13], but also because once introduced in the body, benzoxaboroles may encounter calcium phosphate crystallites (such as those found in bone). Indeed, bone is a tissue in which some drugs, administered for example through oral pathways, can accumulate by adsorption onto bone mineral [14]. This is the case for bisphosphonates like alendronate and tiludronate [15], but also for tetracycline antibiotics like chlortetracycline and doxycycline [16]. Such an accumulation has an impact on the drug release kinetics. In a similar way, in the case of benzoxaboroles, the interaction of the molecules with the crystal surfaces of hydroxyapatites could influence their distribution and determine the drug efficacy. Thus the purpose of this study is to investigate, for the first time, the strength and mode of interaction of benzoxaboroles with hydroxyapatite surfaces.

Below, we will first describe the grafting of benzoxaboroles on ceramic and biomimetic hydroxyapatite phases, discussing in each case, the grafting kinetics, density and mechanism. Using the simplest benzoxaborole (BBzx, Fig. 1a), we determine how this organoboron molecule reacts with calcium phosphate surfaces. Next, in order to understand the potential consequences of benzoxaborole/apatite interactions in a therapeutic context, we will compare the strength and mode of grafting of benzoxaboroles onto hydroxyapatite with other oxo-anionic molecules that are already present in the body and/or more commonly used to functionalize apatite surfaces, like carboxylates and organophosphates. Indeed, carboxylates are widely present in proteins like osteocalcin, which is proposed to bind to bone mineral through peripheral γ-carboxyglutamate moieties [17,18], as well as in small molecules like citrate, whose presence at the surface of bone mineral has been widely investigated in recent years [19]. Organophosphate groups are also exposed at the surface of post-translationally modified proteins like osteopontin, another bone protein known to interact strongly with hydroxyapatite [20]. Finally, we will show that more complex benzoxaborole molecules can also be grafted onto hydroxyapatite, such as the 3-piperazine-bis(benzoxaborole) molecule (bis-pipe-BBzx, Fig. 1d), that displays a high biological activity against bacteria like *Mycobacterium luteum* and fungi like *Candida tenuis* and *Aspergillus niger* [10,21].

2. Materials and methods

2.1. Materials

The 2-hydroxymethylphenylboronic acid cyclic monoester ($C_7H_7BO_2$, benzoxaborole, abbreviated BBzx, Fig. 1a) was purchased from Sigma-Aldrich (97% purity), while the 3-piperazine-bis(benzoxaborole) molecule (abbreviated bis-pipe-BBzx, Fig. 1c) was synthesized according to previously published procedures [22]. Sodium phenylphosphate ($C_6H_5PO_4Na_2$, ABCR, 98%, Karlsruhe – Germany) and benzoic acid (C_6H_5COOH, Acros organics, 99.5%, Geel – Belgium) were used as received.

Ceramic hydroxyapatite was purchased from BioRad (CHT Type II 40 μm, BioRad, Hercules, CA, USA). This phase is referred to as HA_{ceram}. SEM and TEM images show that the powder consists of beads of 20–50 μm diameter, each bead being composed of smaller crystallites of hydroxyapatite (see Fig. S1 in supporting information). Prior to grafting experiments, 30 g of the ceramic HA were suspended in 150 mL of H_2O for 24 h, and then dried at 100 °C for 16 h in vacuum, in order to homogenize the surface properties of the material prior to the grafting. The sample was stored at room temperature. The Brunauer Emmett Teller (BET) specific surface area of this powder, derived from N_2 physisorption at 77 K, was 22 $m^2\ g^{-1}$. XRD, ^{31}P and ^1H solid state NMR confirmed the presence of crystalline HA only.

The biomimetic hydroxyapatite phase, referred to as HA_{nano}, was synthesized following the procedure described by Pascaud et al. [23]. In brief, it was prepared by introducing 100 mL of a calcium nitrate solution ($Ca(NO_3)_2$, $4H_2O$, $[Ca^{2+}] = 0.295\ mol.L^{-1}$) into 200 mL of an ammonium hydrogenphosphate solution ($(NH_4)_2HPO_4$, '[P]' = 0.606 $mol.L^{-1}$). The excess of phosphate ions allowed the buffering of the solution. The suspension was stirred at room temperature for 5 min, and then transferred into a sealed container to mature at 37 °C for 1 day (in absence of stirring). The precipitate was then vacuum filtered, washed 3 times with 100 mL of deionized water, and freeze-dried. Then the powder was sieved (<125 μm) and stored in a freezer. SEM and TEM images show that the powder consists of agglomerated nanocrystals. XRD shows the lower crystallinity of this phase, in comparison to HA_{ceram} (see Fig. S1). The Brunauer Emmett Teller (BET) specific surface area of this powder, derived from N_2 physisorption at 77 K, was 162 $m^2\ g^{-1}$. According to elemental analyses, the Ca/P atomic ratio in HA_{nano} is 1.49 (with $\%_{wt}$ (Ca) = 34.19 and $\%_{wt}$ (P) = 17.76). It is worth noting that all these characterizations are consistent with those reported previously for biomimetic nanocrystalline apatites, in which the crystallites are formed of an apatitic core covered by an amorphous hydrated layer, which is prone to ionic exchanges [23,24].

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