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Textural characteristics of model and natural bone tissues and interfacial behavior of bound water

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ABSTRACT

Water, as a probe liquid bound in model systems (highly disperse hydroxyapatite – protein composites as a model of the main components of bones) and rat bone tissues healthy and affected by osteoporosis occurred due to experimental Alzheimer's disease (EAD), has been investigated using low-temperature ^{1}H NMR spectroscopy, NMR cryoporometry, TG/DTG/DTA, DSC, and TG and DSC thermoporometry. The textural characteristics of these intact systems cannot be studied using the standard adsorption methods, but the cryoporometry and thermoporometry methods give these characteristics. The ^{1}H NMR spectra of water bound in model and natural bone tissues include signals, which can be assigned to strongly associated (typical) water (SAW, chemical shift of proton resonance δ_H = 5–6 ppm) and weakly associated (atypical) water (WAW) at δ_H = 1–2 ppm. Contributions of SAW and WAW give information on textural organization of both model and natural bones. The influence of such co-adsorbates as HCl, $CDCl_3$, CD_3CN, C_6D_6, and $(CD_3)_2SO$ on the interfacial behavior and clustering of bound water depends on their polarity, amounts of components, and textural and structural features of the materials analyzed with the ^{1}H NMR spectroscopy and cryoporometry methods. According to the NMR cryoporometry data, the EAD causes an increase in nanoporosity of the bone tissues. The total porosity and the specific surface area of biostructures (accessible for water molecules and estimated using NMR cryoporometry and TG thermoporometry methods with a model of cylindrical pores) are larger for the EAD sample. Weakly polar chloroform-*d* has a significant influence on the organization of water in the bone tissue, and this effect is greater for the EAD sample as more porous material.

1. Introduction

The structural and textural characteristics play an important role in both biomaterials performance and functioning of such biostructures as bone tissues. The characteristics of the latter can change during specific diseases or in consequence of them. To develop improved biomaterials, deeper insight into regularities of a "structure–property" type is necessary for both model systems (much simpler than natural ones) and natural biotissues that can be affected by different diseases. Therefore, different model composites with nanoparticulate hydroxyapatite and proteins (gelatin, human serum albumin, HSA, bovine serum albumin, BSA) and natural bone tissues healthy and affected by Alzheimer's disease (AD) are analyzed here. Connection between AD, which is the most common form of dementia, and changes in the characteristics of bone tissues and bound water are determined here.

Although the AD course is unique for every individual, there are many common symptoms. As the AD advances, symptoms include confusion, irritability and aggression, mood swings, language breakdown, long-term memory loss, and the general withdrawal of the sufferer as their senses decline [1–4]. The AD can be accompanied by general weakness and a high frequency of bone fracture [5–7]. This is of importance during treatment of the AD patients because they are inclined to fall due to the loss of movement coordination [8]. The AD can increase the probability of osteoporosis [8], which is due to rather changes in living conditions (vitamin deficit, sun light absence because of sedentary life) than the AD *per se* [9,10]. Systematic investigations [10] showed that the vitamin deficit is the main reason of osteoporosis of AD patients. However, it is difficult to study the changes in the bone tissue structure of the AD patients because of a long term of this disease and advanced age of

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the patients. Therefore, a method of experimental stimulation of the AD of rats was developed using a short-term blocking of blood access to brain by squeezing cardiac vessel bundle [11]. This procedure can lead to lethal complications of a part of rats. However, survivor rats demonstrated practically normal vital activity. They can be used to elucidate the changes in bone tissues due to the experimental AD (EAD), term of which corresponds to the major part of their life [11]. This method allows one to compare the characteristics of bone tissues of animals with and without EAD who live under the same conditions.

It was shown previously [12] that changes in structure of human bone tissues affected by osteoporosis, which enhances bone tissue porosity over a broad range of pore sizes, can be analyzed using low-temperature high resolution ^{1}H NMR spectroscopy of static samples using bound water as a probe compound. The data on the temperature behavior of bound water were used in NMR cryoporometry analysis [12–16]. This approach allows us to study changes in bound water state and structure affected by changes in the bone tissue texture [12,13], as well as water bound in different solid and soft materials [14–17]. Typically, bound water includes strongly (SAW, δ_H = 4–5 ppm) and weakly (WAW, δ_H = 1–2 ppm) associated waters. SAW and WAW can correspond to strongly (SBW) (changes in the Gibbs free energy $-\Delta G > 0.5$ kJ/mol) or weakly (WBW) ($-\Delta G < 0.5$ kJ/mol) bound waters. SAW, which is characterized by chemical shift of the proton resonance δ_H = 4–5 ppm close to that of bulk water, forms larger structures (nanodomains) in broader pores than WAW (δ_H = 1–2 ppm). The latter forms clusters, for example, of 1D or 2D types or small 3D or branched 1D/2D/3D, in narrower pores or voids than SAW. The average number of the hydrogen bonds per a molecule is higher for SAW (3–4 bonds per a molecule including two bonds (n_O) with lone-electron pairs of oxygen atom and two bonds with H atoms, $1 < n_H \leqslant 2$ in average) than WAW ($n_H \leqslant 1$). Thus, the WAW molecules form one or less hydrogen bond per a molecule (as a proton-donor) or have the geometry of weak hydrogen bonds with surroundings far from the optimal ones (or dispersion interactions with hydrophobic neighbors) that lead to low δ_H values [14–17]. Osteoporosis is accompanied by a decrease in the amounts of WAW, that is, water clusters and domains (SAW) bound in bone tissues increase in size due to increase in voids in the bone tissues [12].

It is well known that osteoporosis leads to lower bone mineral density [18] and changes bone tissue architecture [19] that can cause a decrease in bone strength and an increase in fracture risk. The changes in bone tissues were studied using different NMR techniques mainly magnetic resonance imaging (MRI) and micro MRI [20–33]. Detailed textural characterization of bone tissues at the nanoscale level can be done using NMR cryoporometry [12–17,34]. Fractal analysis can be used to quantify changes in bone induced through the use of a rat tail-suspension model to simulate microgravity-induced osteopenia [35]. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) can give useful information on the hierarchical structure of bones [36–40]; however, this information is rather qualitative with respect to the bone texture. Measures of mobile and bound water by NMR relaxometry are correlated with bone strength and toughness [41]. However, these data were not used for quantitative textural characterization of bones. Micro-computed tomography and bone morphometry give microstructure of bone [42–49], but some important textural information corresponds to the nanoscale level. The low-temperature ^{1}H NMR spectroscopy data can be used in NMR cryoporometry of different solid and soft materials, including biomaterials to obtain the textural characteristics at the nanoscale level using water, which presents in biosystems, as a probe compound [12–17,34]. This is an invasive method and it can be applied to the initial samples in contrast to many other methods which require specific pre-treatment procedures (drying, degasing, heating, etc.) that can change the textural characteristics of soft materials. Notice that interfacial phenomena (e.g., interactions between organic and inorganic components affecting interactions with bound water) and structural properties (chemical composition, porosity, etc.) can play a key role in bone tissues [12,13] (e.g., changes in them occur due to osteoporosis affecting the bone texture), as well as in model hydroxyapatite composites and related systems [50–59]. Thus, the mentioned NMR techniques can give important information on the interfacial phenomena and textural characteristics of model systems based on hydroxyapatite (as the main mineral component of bones) and bone tissues analyzed in this paper. Notice that the temperature behavior and structure of bound water (e.g., formation of its loose branched clusters or denser nanodomains at the interfaces) used as a probe liquid in NMR spectroscopy and cryoporometry investigations depend on the type (polarity, molecular size, possibility of the formation of strong hydrogen bonds) of dispersion medium, such as air or nonpolar (e.g., CCl_4, C_6H_6, or C_6D_6), weakly ($CHCl_3$ or $CDCl_3$, CH_3CN, or CD_3CN) or strongly (dimethylsulfoxide (DMSO)) polar solvents [14–16]. Therefore, the aim of this work was to study the behavior of water bound in model hydroxyapatite/protein/organic co-solvents systems and natural bone tissues, as well as the effects of experimental AD (EAD) of rats on state of water bound in bone tissue of laboratory animals with EAD in comparison with control ones without EAD. The comparative investigations of model and natural systems were carried out using low-temperature ^{1}H NMR spectroscopy (to determine the temperature behavior of bound water and its structural and thermodynamic characteristics), NMR cryoporometry (giving structure of bound water confined in pores and pore structure), DSC and photon correlation spectroscopy (to characterize model hydroxyapatite/protein systems), TG (giving amounts of desorbed water), and DSC and TG thermoporometry (structure of pores) methods.

2. Materials and methods

2.1. Materials

Samples of nanoparticulate hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), HA, at the specific surface area S = 70–120 m^2/g were synthesized by precipitation from aqueous solutions of salts $Ca(NO_3)_2{*}4H_2O$ and $(NH_4)_2HPO_4$ at high pH values. The 0.2 M solution of $(NH_4)_2HPO_4$ was added in driblets to the $Ca(NO_3)_2$ solution stirred that resulted in precipitation of HA. It was boiled for 10 min, filtrated, twice washed off by hot distilled water, and centrifuged. Then, it was dispersed in acetone, sonicated for 1 min, and centrifuged (6000 rpm) for 20 min. The product was dried in air at room temperature, at 80–110 °C, and then at 240 °C for 1 h. Nanocomposites of HA with gelatin (from porcine skin, Sigma–Aldrich) or/and HSA were prepared using 0.5–1 g of a protein, 2–8 g of hydroxyapatite and 50–100 ml of distilled water, stirred at 40 °C, sonicated for 15 min, and dried at 60 °C for several hours.

The second set of composite samples was prepared with hydroxyapatite precipitated in the presence of proteins then treated at 120 °C (composite) or 900 °C (control sample with pure hydroxyapatite). Composites with proteins were synthesized by simultaneous precipitation of the mentioned salts and proteins in 25% ammonium solution [60] and then dried at 120 °C. The samples have the specific surface area S_{BET} = 70 (hydroxyapatite), 158 (hydroxyapatite/gelatin, HG1), 27 (HG2), and 118 m^2/g (hydroxyapatite/gelatin/BSA, HGA).

Tail vertebrae 2–5 were obtained at autopsy of adult Wistar rats affected by EAD and healthy control samples (i.e., without EAD). The bone tissues were stored at 10 °C in physiologic saline (PS)