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Phosphorus-31 spin–lattice NMR relaxation in bone apatite and its mineral standards

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Abstract

Phosphorus-31 spin–lattice relaxation, both in the laboratory ($B_0 = 4.7$ T) and rotating frame ($B_1 = 2.2$ mT), was studied in the following samples: mineral of whole human bone (samples B1–B6), apatite prepared from bone (BHA), natural brushite (BRU), synthetic hydroxyapatite hydrated (HAh) and calcined (HAc), and synthetic carbonatoapatite of type B (CHA-B) with 9 wt% of CO_3^{2-}. The T_1^P relaxation time was determined directly using the saturation recovery technique, while the $T_{1\rho}^P$ relaxation time was measured via $^1H \rightarrow {}^{31}P$ CP by incrementing the ^{31}P spin-lock. In order to avoid an effect of magic-angle spinning (MAS) on CP and relaxation, the experiments were carried out on static samples. The ^{31}P spin–lattice relaxation was discussed for trabecular and cortical bone tissue from adult subjects in comparison to the synthetic mineral standards. None of the reference materials has matched accurately the relaxation behaviour of the bone mineral.

The most striking differences between the examined substances were observed for T_1^P, which for human bone was sample dependent and appeared in the range 55–100 s, while for HAh, HAc, and CHA-B was 7.2, 10.0, and 25.8 s, respectively. Possible reasons of so large relaxation diversity were discussed. It has been suggested that T_1^P of apatites is to some extent dependent on the concentration of the structural hydroxyl groups, and this in turn is controlled by the material crystallinity. It was also found that T_1^P decreased on hydration by ca. 30%.

For $T_{1\rho}^P$, both its magnitude and dependence on the CP contact time gave useful structural information. The dehydrated samples (HAc and BHA) had long $T_{1\rho}^P$ over 250 ms. Those, which contained water, either structural (BRU) or adsorbed on the crystal surface (HAh, CHA-B, and B1–B6), had shorter $T_{1\rho}^P$ below 120 ms. It was concluded that the effect of water on $T_{1\rho}^P$ is much more pronounced than on T_1^P. The interpretation has involved P-OH groups and adsorbed water, which cover the apatite crystal surface.

Keywords: Bone; Mineral; Apatite; ^{31}P NMR; Cross-polarization; Relaxation

1. Introduction

Bone is dynamic tissue which undergoes a continuous rebuilding process in the course of human life. This process relies on the resorption of old tissue by osteoclasts (bone resorption cells) and the synthesis of new tissue by osteoblasts (bone forming cells). That is why our bones can grow and retain structural integrity through the repair of micro-fractures. At the same time, the bone mineral is a body reservoir of calcium and phosphorus. Many aspects of the bone turnover are still not properly understood, so their elucidation is essential for proper assessment of bone strength (fracture risk) and treatment of bone diseases, such as osteoporosis [1–3].

As concerns the tissue morphology, bone is ca. 20% trabecular (spongy) and 80% cortical (compact). Trabecular bone consists of a network of interconnected plates and struts. Trabecular bone has much higher surface area than cortical bone and therefore it is metabolically more active and responsive to pathological effects. The bone tissue consists of cells (ca. 2 wt%) and extracellular matter. The extracellular material contains respectively ca. 65, 25, and 10 wt% of inorganic constituents (bone mineral), organic constituents (mostly collagen), and water [4]. The bone mineral is formed of very small crystals (large specific

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surface area) deposited on the organic matrix, which is ca. 90% collagen.

The bone mineral is usually identified with calcium hydroxyapatite (HA): $Ca_{10}(OH)_2(PO_4)_6$ [5]. If not superpure, HA crystallises in the hexagonal space group $P6_3/m$ [6]. The structural hydroxyl groups of HA are crystallographically equivalent and located at the edges of unit cells in the—O–H O–H O–H O–H—columns, parallel to the *c*-axis. They do not participate in mutual hydrogen bonding, because the successive oxygen atoms are too distant from each other (3.44 Å). Stoichiometric HA has the molar Ca/P ratio equal to 1.67. Biological apatite is non-stoichiometric, because of extensive ionic substitution of the crystal lattice and abundant adsorption at the crystal surface [7]. In particular, CO_3^{2-} ions can replace OH^- or PO_4^{3-} ions, leading to carbonatoapatites of types A and B, respectively. The bone mineral is always non-stoichiometric calcium apatite deficient in hydroxyl groups [8]. It should be classified as calcium carbonatoapatite of type B (CHA-B) rather than as HA [7,9,10]. The carbonate anion, CO_3^{2-}, is the principal minor constituent of the bone mineral, estimated at 5–8 wt% [4,7,11,12]. The hydroxyl content was estimated at 21% of the HA stoichiometric value [8].

Intact bone is a demanding tissue for structural studies. Serious experimental problems arise from the morphological diversity of bone and from the co-existence, interrelationship, and great complexity of its organic and inorganic components. Furthermore, one has to perform non-invasive analysis, because bone samples are very sensitive to physical effects and chemical treatment. Solid-state ^{31}P NMR gives us a unique opportunity to look specifically at the mineral of whole bone without any chemical pretreatment [13,14], thus avoiding intervention in the bone structure.

We are aware that this work can be of interest for people qualified in medicine or biology, who are not very familiar with NMR relaxation. For them, we present the following brief introduction to the subject. More details can be found in numerous NMR books [15–19]. NMR relaxation is the process of return of a nuclear magnetization to its thermal equilibrium state. This return is mediated by magnetic fields fluctuating with molecular motions. For non-quadrupolar nuclei, these can be either dipolar fields or those originated from chemical shift anisotropy (CSA). The single relaxation rate is proportional to the square of the fluctuating field strength and dependent on the correlation time of the relevant molecular motion. It follows that relaxation significantly declines with an increase of distances between interacting nuclei (dipolar contribution) and becomes faster with larger CSA of relaxing nuclei (CSA contribution). Then, spin–lattice relaxation is dependent on the molecular mobility of the lattice, in which the relaxing spin system is embedded.

For relaxation exponential on time, one can determine a corresponding time constant. Recovery of the longitudinal magnetization in a strong static magnetic field $B_0 = \omega_0/\gamma$ occurs with the time constant T_1, which is called spin–lattice relaxation time in the laboratory frame. The longitudinal magnetization can be spin-locked by a weak radiofrequency field $B_1 = \omega_1/\gamma$, rotating with the frequency ω_1 in the plane normal to the B_0 field. In this case, it decays with the time constant $T_{1\rho}$, which is called spin–lattice relaxation time in the rotating frame.

The T_1 and $T_{1\rho}$ relaxation times are controlled by molecular dynamics occurring on the appropriate frequency scale. The T_1 relaxation is most efficient, when the lattice executes motions in a frequency range close to the Larmor frequency ω_0, so it probes dynamic processes in the MHz range. The $T_{1\rho}$ relaxation is favoured by motions occurring at or near the frequency ω_1, so it probes dynamic processes in the tens of kHz range. Needless to say, the T_1 and $T_{1\rho}$ relaxation times are both temperature dependent and vary with B_0 and B_1, respectively. Relaxation can be significantly modified by magic-angle spinning (MAS) [20], so that care must be employed when interpreting and comparing relaxation times measured under MAS conditions. Overall, the T_1 and $T_{1\rho}$ relaxation times can be specific for studied materials, in particular for biological samples.

Solid-state NMR studies of bone have been reviewed [14]. There were only a few articles devoted to the ^{31}P NMR relaxation studies of bone *ex vivo* in high magnetic fields. The ^{31}P spin–lattice relaxation time T_1^P was found equal ca. 100 s [21,22] and this decreased by approximately 15% from the dried to the fully hydrated sample [21]. In contrast, for synthetic HA, T_1^P was considerably shorter and varied from 1 to 22 s [21,23]. The cited results are for differently prepared samples, different magnetic fields and different MAS rates. Recently, Takata et al. [24] found that T_1^P of male rat femurs increases on age and suggested that it can be used for evaluation of bone quality and strength. Homonuclear ^{31}P–^{31}P spin–spin relaxation in bone, dental enamel, and synthetic apatites was characterized by van Vleck second moments M_2^{PP} [25]. It was found that a significant portion of protonated phosphates (HPO_4^{2-}) is located on the surfaces of biological apatite crystals, and the concentration of unprotonated phosphates (PO_4^{3-}) inside the crystal lattice is elevated with respect to the surface. Then, it was concluded that the total concentration of the surface HPO_4^{2-} ions is higher in the younger, less mature biological crystals. We feel that it is worthwhile to acquire more information on the ^{31}P spin–lattice relaxation in bone and its mineral standards, especially that the rotating-frame relaxation has not been yet investigated.

2. Experimental

Solid-state ^{31}P NMR measurements were done on a Varian Unity Plus-200 spectrometer at 81 MHz, using a Doty double-bearing probe with 7 mm zirconia rotors. The spectra were recorded at 298 K without sample spinning. Cross-polarization (CP) [26] from protons to phosphorus-31 was set and adjusted on calcium hydroxyapatite.