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FTIR STUDY OF BIOLOGICAL HYDROXYAPATITE

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Abstract

In this study Fourier transform infrared (FTIR) spectroscopy was used to resolve setting of carbonate ions in biological hydroxyapatite (BHAp). Even though preliminary XRPD results indicate that BHAp is B-type carbonated hydroxyapatite, the detailed FTIR spectroscopy analysis show that carbonate ions substitute both phosphate and hydroxyl ions in the crystal structure of BHAp, representing a mixed AB-type.

Introduction

Comprehensive studies of natural minerals from bones show that they are not a pure hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, HAp); they are associated with elements such as CO_3^{2-} , HPO_4^{2-} , Na^+ , Mg^{2+} , Sr^{2+} , K^+ , Cl^- and F^- [1]. On the basis of the substitution sites carbonated apatites are classified to: A-type where CO_3^{2-} ions occupy the OH^- sites; B-type where CO_3^{2-} ions occupy the PO_4^{3-} sites, and AB-type where they occupy both OH^- and PO_4^{3-} sites [2].

In the 1960's the crystal structure of HAp based materials is determined in details [3]. Until now, crystal structure of carbonated hydroxyapatites (biological, natural minerals, as well as synthetic ones), has been examined in a lot but still remained insufficiently clarified due to the inability to precisely determine the position of the carbonate ions directly based on diffraction data. The main difficulty to unambiguous determination of the carbonated hydroxyapatites structure from XRPD data is very small crystallite size and low crystallinity. However, vibrational spectroscopy techniques, both Raman and FTIR, are irreplaceable for study the nature of carbonate occupancy in the crystal structure of the carbonated apatite. According to the position of the vibrational bands in FTIR and Raman spectra, the occupancies of carbonate ions can be precisely determined; is they A-, B-, mixed AB-type, or an unstable (i.e. surface) carbonate locations [4].

It is found that hydroxyapatite from bone contains from 2.3 to 8 wt% of carbonate ions [4]. The carbonate ions have a large effect on the nature of BHAp; they increase solubility in acids and retard a crystal growth. Since a composition and crystal structure of bone are influenced by age and health status of individual, of the most interest is to determine the type of carbonated apatite in concrete natural bone, at first for the fundamental knowledge of bone regeneration, and further for the design a biocompatible synthetic carbonate-substituted hydroxyapatite appropriate for reparation of a bone defects.

Here, we chose a biological carbonated apatite extracted from human alveolar bone as a model system to resolve the problem of carbonate ions positioning using FTIR spectroscopy technique.

Experimental

The biological apatite (BHAp) was derived from human alveolar bone. To remove organic part, the bone was deproteinated, after that, the sample was dried at room temperature and grinded in an agate mortar.

The crystal phase of BHAp was identified by XRPD analysis obtained on Philips PW-1050 automatic diffractometer. The diffraction measurement was done over scattering angle from 8 to 120 °2 θ with a step of 0.02° and a counting time of 12s. The FTIR measurement was performed on MIDAC M 2000 Series Research Laboratory FTIR Spectrometer using the KBr pellet technique, in the spectral range of 400–4000 cm⁻¹, with spectral resolution of 4 cm⁻¹. The deconvolution of recorded FTIR spectrum was done using PeakFite™ (version 4.05, demo) software.

Results and Discussion

Preliminary results of XRPD analysis show that the BHAp has a poorly crystalline hydroxyapatite phase of B-type.

The FTIR spectrum of BHAp (Fig. 1a) has four typical apatite phosphate (PO₄³⁻) modes: triple degenerative ν_3 asymmetric stretching mode characterized by a strong, complex band in the 1000-1150 cm⁻¹ region; a medium intensity band at 961 cm⁻¹ due to ν_1 symmetric stretching vibration; double degenerative ν_4 bending vibration with bands at 564 and 603 cm⁻¹, and weak band near 470 cm⁻¹ corresponds to double degenerative ν_2 bending vibration. The vibrational band of the water appears at 1635 and 3430 cm⁻¹; and that of OH⁻ libration modes at 635 cm⁻¹. In addition to the phosphate and hydroxide bands of pure HAp, the spectrum of BHAp also shows additional bands. The carbonate bands appear at 755 cm⁻¹ for the ν_4 stretching mode, band at 872 and shoulder at 880 cm⁻¹ are due to ν_2 stretching mode, while bands at 1418, 1456, 1506 and 1558 cm⁻¹ are attributed to ν_3 stretching mode. The bands at 872, 1418 and 1456 cm⁻¹ suggest the B-type of carbonated apatite, while the bands at 880 and 1558 cm⁻¹ are characteristics of the A-type [4]. These results confirmed that the hydroxyapatite from the human alveolar bone is mixed AB-type carbonated apatite.

It is known that the ratio of the intensities of the peaks at 880 and 873 cm⁻¹ corresponds to the ratio of A-type:B-type carbonated apatite. Therefore, the spectral region 860-890 cm⁻¹ was re-examined; after a deconvolution it is found that $I_{880}/I_{872} = 0.32$, indicating that much more carbonate ions are placed on the phosphate (B) site than on the hydroxyl (A) site in the crystal structure of BHAp. The 860-890 cm⁻¹ band (recorded, deconvoluted and fitted) is presented in Fig. 1b.

Besides, absorption band at 1658 cm⁻¹ is assigned to the amide I band, while bands appear at 2850 and 2950 cm⁻¹ belonging to the aliphatic C–H stretching vibrations. These bands point to some organic impurities mixed with the BHAp, must probably of collagen – which is the main organic component of bone.

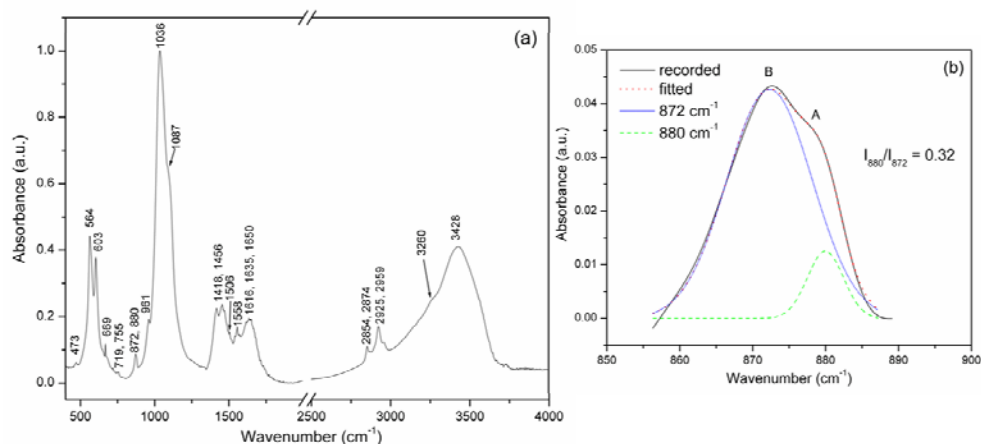


Fig.1 (a) FTIR spectrum of BHAp, and **(b)** recorded, deconvoluted and fitted 860-890 cm^{-1} spectral region.

Conclusion

A biological carbonated hydroxyapatite (BHAp) extracted from human alveolar bone is chosen as a model system to resolve the problem of crystal structure and carbonate ions placing. Rietveld refinement of XRPD data confirmed that BHAp has a crystal structure of hydroxyapatite with the B-type of carbonated hydroxyapatite.

The FTIR results show that BHAp contains small amount of organic part of bone tissue, must probably of collagen. Furthermore, FTIR analysis resolved the nature of carbonated apatite. According to the results carbonate ions are placed in both A and B positions, which indicates mixed AB-type of carbonate apatite. After the deconvolution of FTIR band at 860-880 cm^{-1} it is calculated that $I_{880}/I_{872} = 0.32$, meaning that only a small amount of carbonate ions occupy the OH^- sites while a large amount is at PO_4^{3-} positions. Hence, the BHAp from human alveolar bone is mixed AB-type of carbonated hydroxyapatite but with dominant B-type.

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