



IR and Raman micro-spectroscopy applied for studying structural characteristics of dental apatite

Използване на ИЧ и Раманова микроспектроскопия за изследване на структурни характеристики на дентален апатит

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Introduction

Biological apatite formed under physiological conditions differs from high temperature geological apatite in many characteristics. Vibrational micro-spectroscopy is a powerful tool for studying local atomic structure, sensitive to chemical and structural inhomogeneity, phase impurities, isomorphic substitution, degree of structural disorder and crystallographic orientation. The purpose of this study is to apply different vibrational spectroscopic techniques to probe the structural state of dental apatite, namely enamel and coronary dentine.

Material and methods

Twenty-four cross-sections from 6 teeth were prepared by microtome Leica 1600. The orientation of the slices was parallel and perpendicular to the elongation of the apatite rods building enamel. One side of the plates was polished subsequently with 3, 1 and 0.1 μm silica (SiO_2) polishing suspensions. Infrared (IR) spectra were measured using Bruker FT-IR spectrometer Tensor 37 and IR microscope Hyperion 2000, equipped with MCT LN cooled detector. The spectra in reflection mode were collected using 15 X Cassegrain objective in the range 400–4000 cm^{-1} . The ATR (Attenuated Total Reflection) spectra were measured using Ge-ATR x20 objective in the range 600–5000 cm^{-1} . All the spectra were collected from the area with size 100×100 μm with spectral resolution of 4 cm^{-1} , averaged over 72 scans. Data acquisition was performed using OPUS 6.5 software (Bruker). Raman spectra were recorded in backscattering geometry using HORIBA JobinYvon Labram HR spectrometer, Olympus BH2 microscope, 633-nm line of He-Ne laser, CCD detector, and x100 objective. Origin 9.0

software package was used for spectral evaluation. Scanning electron images in regimes of secondary electrons (SEI) and backscattered electron contrast (BEC) were obtained using JEOL JSM 6390 electron microscope operating at 20 kV accelerating voltage.

Results and discussion

The IR reflection spectra (Fig. 1a) collected along the profile from the surface of the teeth to dentine reveal a gradual decrease of the intensity and change of the intensity ratio between the two most intensive peaks at 1050 and 1095 cm^{-1} , resulting from antisymmetric stretching vibrations of phosphate group ($\nu_3 \text{PO}_4$). These peaks are slightly shifted in the spectrum of dentine. Such spectral variations may be associated with increase of structural disorder and decrease of the size of crystallites toward dentine. The main reason for increase of structural defects in biological hydroxyapatite is the isomorphic substitution of carbonate for phosphate group, known as B-type, and carbonate for hydroxyl group, known as A-type carbonate substitution (Penel et al., 1998). The presence of B-type carbonate substitution is evidenced by the peaks at 874, 1450 and 1470 cm^{-1} caused by $\nu_2(\text{CO}_3)$ bending and antisymmetric $\nu_3(\text{CO}_3)$ stretching of carbonate group, respectively (Fig. 1a, b). It has been shown, that antisymmetric stretching of phosphate group in apatite is most sensitive to the degree of carbonate substitution (Wopenka, Pasteris, 2005), and thus the change of the intensity ratio of the two characteristic peaks at 1050 and 1095 cm^{-1} ($\nu_3 \text{PO}_4$) is most probably related to degree of B-type carbonate substitution. To check this assumption Raman spectroscopy was applied, because according to the selection rules, symmetric stretching modes should be better resolved. An increase of the peak at 1071 cm^{-1} (ν_1 B-type CO_3) with decrease of

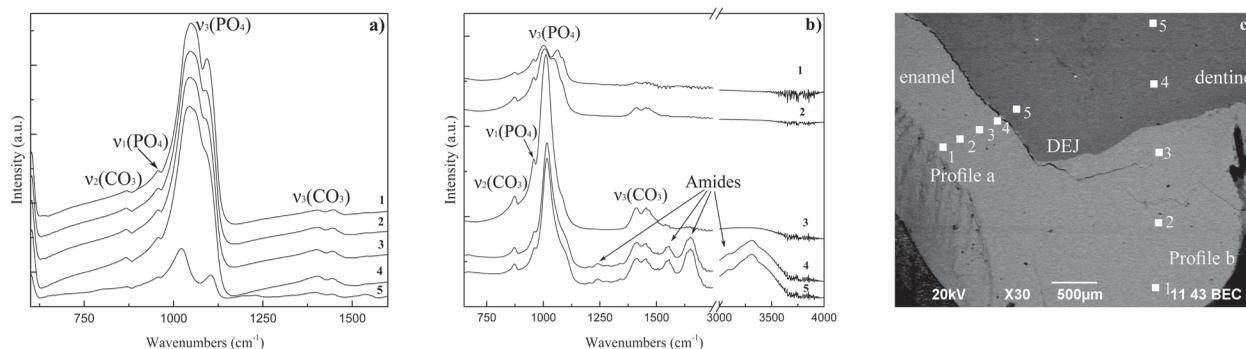


Fig. 1. *a*, IR reflection spectra collected along the profile **a** (see Fig. 1c) from the surficial enamel (1) to dentine (5); *b*, ATR IR spectra collected from the profile **b** (see Fig. 1c) from the surficial enamel (1) to dentine (4–5); *c*, BEC image of cross section of tooth with indicated profiles, along which the measurements were taken

that at 1048 cm^{-1} ($\nu_3\text{ PO}_4$) confirmed that carbonate for phosphate group substitution in the enamel apatite gradually increases from the surficial layer to dentine. Because of the different mechanism of obtaining ATR spectrum, through so called evanescent wave, the intensity and position of phosphate peaks are changed and direct comparison with infrared reflection spectra cannot be done. On the other hand, when comparing only ATR spectra obtained from the surface enamel to dentine (Fig. 1b), it is seen that the spectra of dentine are more intensive due to better contact of Ge ATR crystal and better penetration depth in softer material. Thus varying amount of organic compound could be detected by the peaks of amides at 1665 , 1552 and 1240 cm^{-1} (Fig. 1b).

Conclusions

The IR reflection and Raman spectroscopy (633 nm excitation wavelengths) are more appropriate tech-

niques for studying enamel, while for dentine better results are obtained using ATR IR micro-spectroscopy. Due to the gradual change of the enamel apatite we should consider the whole profile from the surface to the dentine-enamel junction when studying the effect of various dental treatments.

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