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Multi-frequency electron paramagnetic resonance study of irradiated human finger phalanxes

M. Zdravkova[a], G. Vanhaelewyn[a,1], F. Callens[a,*], B. Gallez[b,c], R. Debuyst[b]

[a] *Ghent University, Department of Solid State Sciences, Krijgslaan 281-S1, B-9000 Gent, Belgium*
[b] *Laboratory of Biomedical Magnetic Resonance, Université catholique de Louvain, UCL, Avenue Hippocrate 10, B-1200 Brussels, Belgium*
[c] *Laboratory of Medicinal Chemistry and Radiopharmacy, Université catholique de Louvain, UCL, Avenue Mounier 73, B-1200 Brussels, Belgium*

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Abstract

Electron paramagnetic resonance (EPR) is often used in dosimetry using biological samples such as teeth and bones. It is generally assumed that the radicals, formed after irradiation, are similar in both tissues as the mineral part of bone and tooth is carbonated hydroxyapatite. However, there is a lack of experimental evidence to support this assumption. The aim of the present study was to contribute to that field by studying powder and block samples of human finger phalanxes that were irradiated and analyzed by multi-frequency EPR. The results obtained from bones are different from the ones obtained in enamel by several respects: the ordering of the apatite crystallites is much smaller in bone, complicating the assignment of the observed CO_2^- radicals to a specific location, and one type of CO_3^{3-} radical was only found in enamel. Moreover, a major difference was found in the non-CO_2^- and non-CO_3^{3-} signals. The elucidation of the nature of these native signals (in bone and tooth enamel) still represents a big challenge.
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1. Introduction

Electron paramagnetic resonance (EPR) spectroscopy is often used in dosimetry, dating and detection of irradiated food where teeth and bones are the most studied biological materials [1]. The measurements are generally conducted in X-band (9.5 GHz) for practical reasons, like the sample size, the spectrometer availability, cost, etc. It was recently suggested to use L-band (1 GHz) EPR spectroscopy for non-destructive dosimetry of whole teeth and fingers [2–4]. Low-frequency microwaves are indeed less absorbed by water and penetrate more deeply into the tissues which make them of use for in vivo measurements. The low frequency has, however, an inconvenience, i.e. the compression of all EPR signals which results in a single line. In this respect higher frequency EPR studies at Q-band (34 GHz) and W-band (94 GHz) are useful for increasing the spectrum resolution and for distinguishing between the individual components in the X- or L-band spectra.

Tooth and bone are both composed of a mineral part (carbonated hydroxyapatite), water and an organic part, but the relative contributions of these three constituents differ in enamel, dentin and bone. Average values are summarized in Table 1 [5]. The EPR stable CO_2^- dosimetric signal can originate from CO_3^{2-} ions, CO_2 molecules or COOH groups present in the calcified tissue structure [6,7]. Tooth enamel is the most sensitive EPR dosimeter due to its high mineral content and degree of crystallinity. Moreover, enamel does not undergo remodelling as dentin and bone do. Bone is esti-

* Corresponding author. Tel.: +32 9 2644352; fax: +32 9 2644996.
E-mail address: freddy.callens@ugent.be (F. Callens).
[1] Postdoctoral Fellow of the Fund for Scientific Research, FWO, Flanders, Belgium.

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Table 1
The main constituents of sound human tooth enamel, dentin and bone (average wt.%) and comparison of the average density of these tissues[a]

Constituent	Enamel	Dentin	Bone
Water	3	10	25
Organic matter	1	20	25
Mineral	96	70	50
Average density (g cm^{-3})	2.92	2.51	2.35

[a] Data from Driessens and Verbeeck [5].

mated to be around 10 times less radiosensitive than enamel [8]. In addition, its bigger organic part could reveal additional radicals. Nevertheless, bone is sometimes the only dosimetric material available. It is actually already studied since several decades (modern and ancient bones from different animals and men [1] (and references therein) [9]), but still needs further analyses. Recently very interesting applications appeared related to human bones exposed for radiotherapy [10] and radiation accidents [8,11].

In human tooth enamel, the EPR spectrum normally consists of a broad native signal supposed to be of organic origin at $g = 2.0045$ and stable CO_2^- signals with g values in the region 2.003–1.997. This signal was thought for a long time to be due to the CO_3^{3-} radical [12], but was later attributed to CO_2^- [13–15]. Thanks to multi-frequency EPR and electron-nuclear double resonance studies, several CO_2^- species differing in symmetry (isotropic, axial, orthorhombic) and location (phosphate or B-site, surface site, occluded water) could be identified. Similar studies with enamel (powders and blocks) heated at 400 °C before irradiation revealed the presence of different CO_3^{3-} radicals, which even become predominant at low microwave power. Other radicals, such as CO_3^-, CO^- and O^- can also be formed [16–20]. The decomposition of both CO_3^- and CO_3^{3-} radicals might produce CO_2^- species [21,22]. The thermal stabilities of CO_3^- and CO_3^{3-} radicals vary from sample to sample depending on their impurity content, pretreatment, etc. Typical values range from several days to 100 days [23].

As the main component in the mineral part of both bone and tooth enamel is carbonated hydroxyapatite, the radicals formed after irradiation can be expected to be similar in both compounds. The present work tries to verify the pertinence of this assumption. Bone powders and blocks from dry human finger phalanxes were irradiated and analysed by multi-frequency EPR, with or without heating before or after irradiation.

2. Experimental

2.1. Materials

Several dry human finger phalanxes of the same hand were supplied from anonymous collection of the Human Anatomy Department of the Faculty of Medicine, UCL. The bones were boiled for 3–4 days, scraped with bistouries, cleaned with grease-removing soap, dichloroethane, rinsed and dried in air. These samples were already used in a previous work [4].

Bone powder was obtained by grinding the irradiated bones with an agate mortar. Powders from the extremities of the phalanx and from its central part yielded identical EPR spectra. Several blocks (with dimensions 1 mm × 1 mm × 3 mm) were cut from the bone prior to irradiation for rotation in mutually orthogonal planes (to be explained in more detail below). Some samples were heated for several hours at 150 °C and other samples at 400 °C for 2–3 weeks prior to irradiation, others were heated after irradiation. For in situ X-band measurements, a Bruker ER4114HT high temperature cavity was used.

2.2. Irradiation

X-irradiations were carried out at room temperature (RT) with a tungsten anticathode Philips X-ray tube, operated at 60 kV and 40 mA. Typical irradiation times of a few minutes were applied with a dose rate of ∼1.3 kGy/min. One phalanx was irradiated with ^{60}Co gamma rays at RT at a dose of 1400 Gy with a dose rate of ∼5 Gy/min. No effect of the type of irradiation was observed.

2.3. EPR spectra

The X-band EPR spectra were recorded at RT using a Bruker ESP300 X-band spectrometer with a maximum available microwave power of 200 mW. The magnetic field and the microwave frequency were measured using a Bruker ER035M Gaussmeter and a HP5350B microwave frequency counter. The Q-band spectra were recorded at RT using a Bruker Elexsys E500 Q-band spectrometer with a maximum available microwave power of 150 mW. The magnetic field and the microwave frequency were measured using a Bruker ER035M Gaussmeter and a EIP 548B microwave frequency counter. In X- and Q-band DPPH ($g = 2.0036$) was used for absolute g-value calibration. The sample rotation for the angular variations was carried out with using a Bruker standard goniometer. The W-band spectra were recorded using a Bruker Elexsys E600 spectrometer. For an absolute g-value calibration, Mn^{2+} in CaO was used and proportionality was assumed between the current through the superconducting coil and the magnetic field.

3. Results and discussion

3.1. Powder spectra

The X-band spectrum of non-irradiated crushed human phalanxes exhibits a broad native signal around $g = 2.0045$, which is however more complex than in human enamel with peaks around $g = 2.007$ and 1.998, and already a small CO_2^- component (see Fig. 5 in Zdravkova et al. [4]).

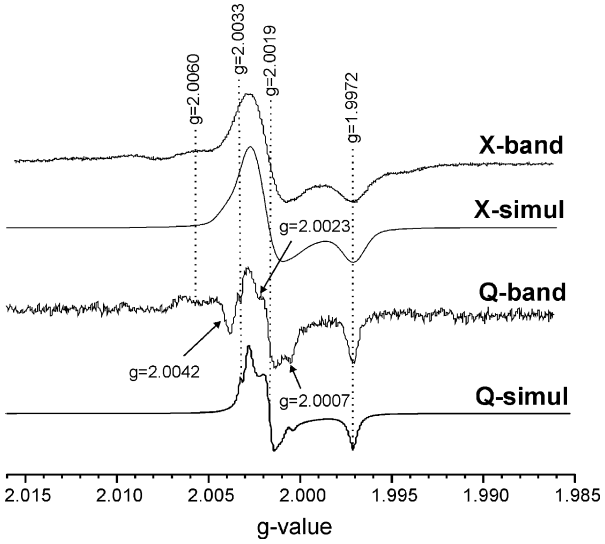

Fig. 1. RT EPR spectra of irradiated bone powder (^{60}Co-dose 1.4 kGy, 3 weeks after irradiation) in X- and Q-band, and simulated spectra using an orthorhombic CO_2^- radical, an axial CO_3^{3-} radical (CO_3^{3-} (2)) and an isotropic signal at $g = 2.0007$. Nearly identical spin Hamiltonian and line width parameters were used for both simulations (e.g. g_x differs by 0.0001 between both bands) and are given for Q-band in Table 2. $P = 0.53$ mW, MA = 0.1 mT.

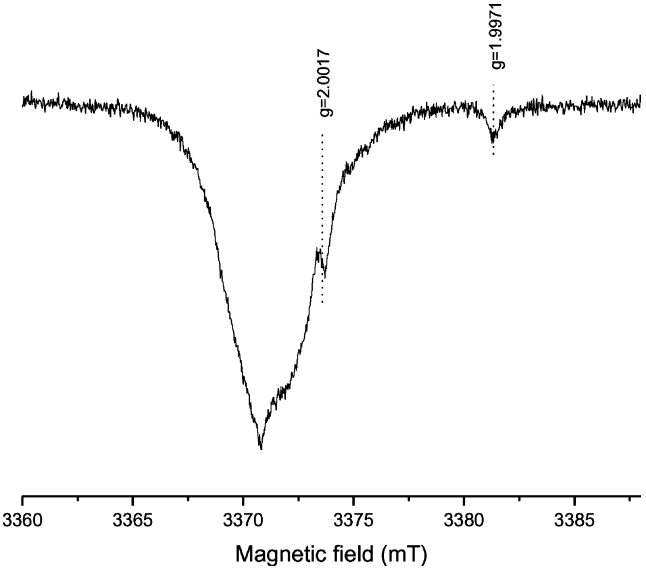

Fig. 2. RT W-band spectrum of irradiated bone powder (X-ray dose 1.5 Gy, 5 days after irradiation). The g-values of the CO_2^- radical are shown. $P = 14$ μW, MA = 0.1 mT).

Irradiation strongly enhances the CO_2^- signal. In X-band this signal looks axial with features at $g = 1.9972$ and 2.0019. The native signal seems distorted and other small components appear on both sides of the dominant CO_2^- signal. Fig. 1 compares X- and Q-band spectra of a sample recorded 3 weeks after irradiation. The Q-band spectrum shows better resolved components at $g = 2.0007$, 2.0023, 2.0033, 2.0042 and 2.0060. The lines at $g = 2.0033$, 2.0042 and 2.0060 are easily saturated and already at 4 mW the $g = 2.0033$ shoulder is no longer visible. The latter could be due to CO_3^{3-} radicals which have principal g-values in this range and which are known to saturate easily. Simulations of the central part of the X- and Q-band spectra are also presented in Fig. 1. Only an isotropic ($g = 2.0007$), an orthorhombic CO_2^- radical (in Q-band the shoulder around 2.0019 is completely resolved) and an axial CO_3^{3-} radical appeared necessary to reach an excellent agreement with experiment (ignoring the unidentified low field features). The g-values and line widths used in the simulations are reported in Table 2. The W-band spectra are characterized by a dominant broad signal at $g \approx 2.06$ and a strong minimum at $g = 2.001$ which can be attributed to an O^- species [17]. In the $g = 2.00$ region only one relatively small CO_2^- signal is well visible (Fig. 2). The poor detectability of the latter signal can be (partly) explained by the large spread in magnetic field in W-band and the overlap with the oxygen spectrum, which is probably enhanced by the heating of the bones during the preparation procedure. The O^- spectrum is also found in Q-band at higher power, albeit less pronounced than in W-band. The fact that the O^- signal has not been detected at all in X-band is more or less compatible with the above, but the underlying reason is for the moment totally unclear.

Table 2
Parameters of the EPR components used for simulations

	g_x	g_y	g_z	Line width (mT)	Shape
CO_2^-	2.0031	1.9972	2.0019	0.20	Lorentzian
CO_3^{3-} (1)	2.0046	2.0034	2.0017	0.16	Gaussian
CO_3^{3-} (2)	2.0040	2.0040	2.0018	0.16	Gaussian

The X-, Q- and W-band results presented above seem quite compatible with the ones reported earlier for unheated tooth enamel. Even in W-band only one (anisotropic) CO_2^- center can be detected. Thus only by comparison with block spectra [16,19] and spectra of heated enamel, it can be expected that the further substructure of the CO_2^- and CO_3^{3-} spectra will become clear. Minor differences between both calcified tissues concern the signals at low field (related to collagen radicals?).

3.2. Blocks of bone

Due to the anisotropic ordering of the apatite microcrystallites, intact bone is also a partially ordered system like tooth enamel. However, it is known that this anisotropy is smaller than for enamel. In order to further investigate this matter and to try to use this anisotropy to our advantage, next to powders, also small elongated bone blocks (plates) were prepared. Assuming that the blocks are rectangular, the following axes system can be defined. The long axis of the phalanx (corresponding to the collagen fiber axis) is labeled z. When the axis perpendicular to the macroscopic surface of the bone is denoted by x, the third orthogonal axis corresponds with y. Blocks were cut for rotation of the magnetic field in the xy and

xz (or equivalently, the *yz*-plane, see below) planes. The rotation with the magnetic field in the *xy* plane does not show any angular dependence of the EPR signals, making the *xz* and *yz* planes equivalent. The spectra are identical to the powder spectra independently of the time after the irradiation.

A small effect of the rotation is observed in the *xz*-plane when freshly irradiated bones are used. During the rotation, the position of the signals does not change but the line width and the relative intensities are affected. These changes are more visible in the region where contributions from CO_3^{3-} are expected (around $g = 2.0033$, see Fig. 3). Small changes are also registered in the dip at $g = 1.9971$ (corresponding to g_y or $g_{\|}$ for CO_2^-).

For tooth enamel, there are several articles in the literature about the effect of the rotation on the EPR spectra of blocks [6,16,19,24,25]. The experiments show a pronounced angular dependence of the EPR spectra for these samples in the planes containing the *x*-axis as defined above. The bone and tooth enamel mineral part are both mainly made up of hydroxyapatite microcrystals, but these crystals are much smaller in the case of bone and also the crystallinity is worse. For example, the average crystallite length decreases from 500–600 nm in enamel to 100 nm in dentin and 30–100 nm in bone, whereas the amorphous phase in bone can reach 40–45% [5]. Up to now the angular dependence in bone is less studied than in tooth enamel. A weak angular dependence in Q-band is reported for a cortical femoral bone after thermal annealing at 200 °C [26]. These authors showed that there are a factor of 2.2 more crystals in the fiber direction than perpendicular to it. Brik et al. [6] observed that the anisotropy in bones from rat legs substantially differs from that of tooth enamel. They found that the oxygen–oxygen axis of CO_2^- in the bones is primarily parallel to the gravitational field of the Earth.

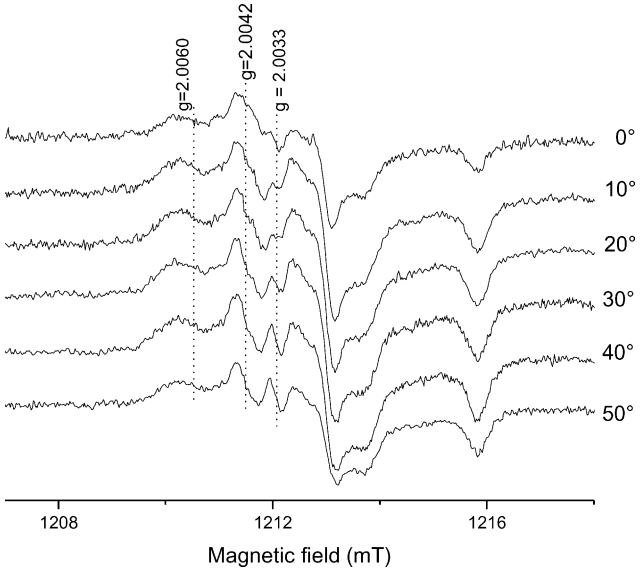

Fig. 3. RT Q-band angular variation of an irradiated bone block performed in the *xz* plane immediately after irradiation (X-ray dose 1.3 kGy). $P = 0.34$ mW; MA = 0.3 mT.

From our investigations the earlier described weak anisotropy of the EPR spectra in bones seems to be completely confirmed. Next to the arguments given above, our results could point to the predominance of "chaotic" CO_2^- radicals in the irradiated, unheated bone. The spectra due to such radicals do not depend on the orientation and are in fact powder spectra. For enamel Brik et al. [7] suggested that these radicals have mainly CO_2 from the nanocapillary water layers, present between the enamel prisms, as precursors. A second possibility consists in carboxyl groups of surrounding amino acids. As also stressed by the same authors, bulk radicals in enamel (and maybe also in bone) are probably for a major part non-CO_2^- radicals, as e.g. CO_3^{3-} and the less stable (in unheated apatites) CO_3^-. This could explain the small variations in the CO_3^{3-} region in Fig. 3. The data are however insufficient to retrieve any information about the orientation of the radical axes system with respect to the apatite crystallites and/or the global bone structure. If the above hypothesis is true, one could a priori hope for a better visible anisotropy in the block spectra of (pre)heated bone (see below). Indeed, if comparable results as for enamel are found, one would expect the "chaotic" part, which largely masks possible anisotropy, to decrease or even to disappear completely, depending on the temperature and the duration of the applied heating.

3.2.1. Heating before irradiation

Different thermal treatments of the samples in air before or after irradiation were performed during this work. On the one hand, heating after irradiation allows to remove the unstable centers but can also produce additional paramagnetic centers. On the other hand, heating before irradiation can sometimes lead to important structural alterations and affect the precursors of the radicals. For example, a thermal treatment at 400 °C during 2 or 3 weeks until constant weight of the sample, as already used in Callens et al. [16] and Sadlo et al. [17,18], is normally enough to deproteinate the bone and thus eliminate the EPR signal due to the collagen content [27]. According to the latter authors, heating at a temperature below 500 °C does not affect the structure and distribution of the mineral phase, although collagen is no longer present in the samples. Treatment of bone at temperatures exceeding 500 °C causes complete disruption of the tissue architecture and the reorganization of the mineral phase into tightly packed, dense crystals. Similar observations are quoted in Gilinskaya and Zanin [23] and Bachmann et al. [28]. According to Gilinskaya and Zanin [23], heating causes removal of adsorbed water (<200 °C), of organic substance (around 350 °C), of bound water (400–600 °C) and of carbonate ions (600–900 °C). Annealing at 400 °C leads to a significant decrease in the CO_2^- content following the removal of part of the bound water stabilizing the CO_2^- radical. Similar statements were made by Brik et al. after their detailed study on the effect of (pre)heating on the magnetic resonance spectra of tooth enamel [7].

Some samples heated at 150 °C for several hours before irradiation did not show EPR spectra different from the ones for non-heated samples. This result is essentially in agreement with the fact that the bones were already boiled during their preparation (see Section 2.1).

Most samples were heated at 400 °C during 2–3 weeks before irradiation. In agreement with the literature, the heated but non-irradiated samples still show a very weak signal around $g = 2.004$. Heating by itself is known to produce EPR signals. Progressively stronger (partially decaying) EPR signals at $g = 2.0043$ with a line width of 0.44 mT were recently observed in bovine dentin after thermal annealings from 100 to 300 °C [28].

Because CO_2^- and CO_3^{3-} spectra are known/expected to be best visible at high and low power respectively, angular variations at different power levels have been performed. Also comparable powder spectra have been recorded.

In both X- and Q-band, the high power powder spectra always reveal a well detectable, albeit substantially decreased, contribution from CO_2^-, mainly based on the presence of the signal around $g = 1.997$, whereas at lower power this signal is practically absent. This means that at least one type of CO_2^- radical is resistant to the temperature treatment. Around 0.3 mW, the spectra are dominated by CO_3^{3-} contributions, as will be discussed in more detail below. As the powder spectra of "chaotic" and anisotropic CO_2^- ions, if both present, are hardly distinguishable even in Q-band, block spectra are indispensable to gain insight into the further substructure of the CO_2^- signal (see e.g. [16] for an enamel study). Like for unheated blocks, hardly any anisotropy could be detected for the CO_2^- signals. Since we could not record a high power block spectrum without a 1.997 component, the conclusion is not as simple as in the enamel case. Only if we assume that the "chaotic" component has disappeared by the preheating, as was the case for enamel, only then we may conclude that also in bone a bulk CO_2^- component is present. We would then have to conclude that the ordering of the apatite crystallites in bone is very weak or even isotropic for the specific samples under study. This would, of course, then also a fortiori explain the very weak anisotropy of unheated bone samples.

Focusing now on the low power spectra, it is found that the EPR signal of an irradiated preheated sample is sharper than that of a non-preheated one. The analysis of the spectrum reveals the presence of a very small quantity of CO_2^- and a much higher quantity of two types of CO_3^{3-} radicals. In Fig. 4 it is shown that the spectrum can be essentially reproduced by adding two CO_3^{3-} spectra. In spite of intensive efforts, some features of the experimental spectrum could not be reproduced in the simulation. Also attempts with three CO_3^{3-} radicals, which were successful for tooth enamel, failed for bone. The two components found in bone correspond with the Z1 and Z2 signals in tooth enamel [20]. The reason why the third enamel component is absent in bone is not clear for the moment. The signals of CO_3^{3-} decay nearly completely in 10 days, but are easily restored by a new irradiation.

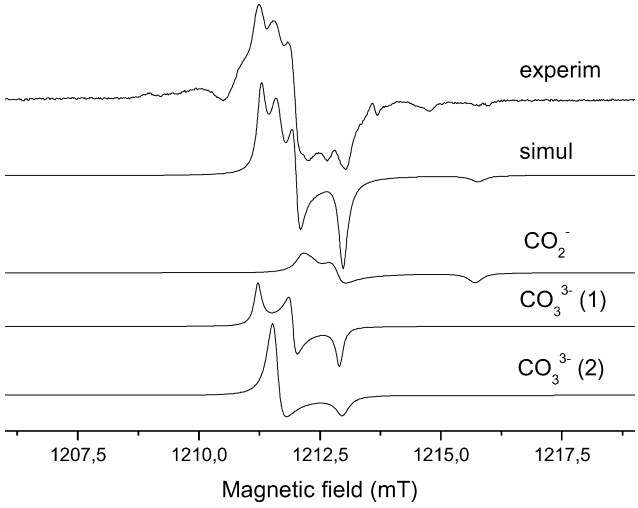

Fig. 4. RT Q-band spectrum of bone powder heated at 400 °C before irradiation (X-ray dose 1.5 kGy) and simulated spectrum with one CO_2^- and two CO_3^{3-} radicals (EPR parameters given in Table 2). $P = 0.33$ mW, MA = 0.1 mT.

Another signal which is probably the isotropic septet due to the isopropyl radical with $g = 2.0038$ and $A = 2.17$ mT [29] is also observed, as well as in post-heated samples (see below). This signal is known to be enhanced by heating up to 250 °C but is lost around 280 °C [30]. The presence of the components at $g = 2.0042$ and 2.0060 after such drastic heating rejects the hypothesis of an organic origin. A carbon centered radical could be suggested like, e.g., CO^- which has g-values (2.0057, 2.0043 and 2.0021 in Callens et al. [31]) in the right range. This assignment is however rather speculative. E.g., CO_3^- radicals, also with g-values in this range, could be considered too, as it is known that heating stabilizes these radicals in synthetic apatites [32].

The above results show that bone behaves quite differently from tooth enamel. If the "chaotic" radical precursors leave in a similar way as in tooth enamel by the heating procedure, we should conclude that the crystallite distribution is nearly isotropic. If the precursors are not (all) destroyed by the heating, it is possible that the CO_2^- signal is non-composite and completely "chaotic". There are also other differences: the third CO_3^{3-} component is either missing or different from the one in enamel and again there are differences in the "native" signals.

3.2.2. Heating after irradiation

A 1.4 kGy gamma-irradiated sample was stepwise heated in air from 100 to 210 °C several weeks after irradiation. After each annealing period of 20 min at the selected temperature, the spectrum was measured at RT. The results obtained using the X-band high temperature cavity are presented in Fig. 5. A decrease of the intensity of the CO_2^- signal and an increase of a signal around $g = 2.0050$ is observed, gradually replacing the $g = 2.0060$ and 2.0042 features from Fig. 1. The major changes in the spectra occur at temperatures higher than 150 °C. At the same time other signals appear and be-

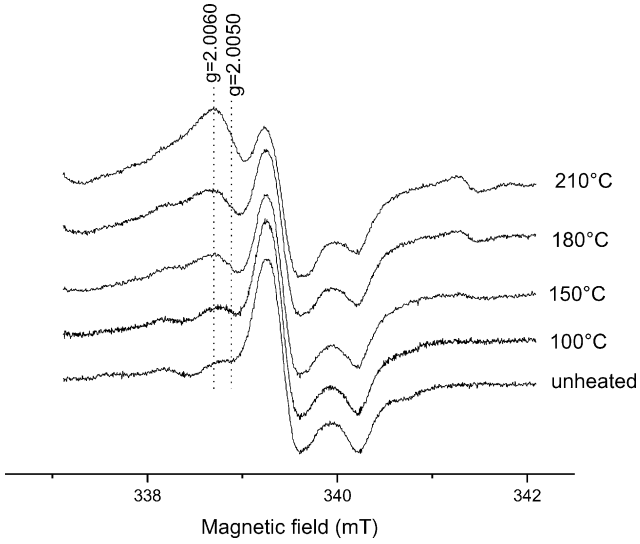

Fig. 5. RT X-band spectra of irradiated bone powder (^{60}Co-dose 1.4 kGy, 2 months after irradiation), heated 20 min at different temperatures. P = 0.32 mW, MA = 0.1 mT.

come more intense with increasing temperature. One of these signals is the already mentioned septet.

Another sample was studied similarly in X-band immediately after a 1.5 kGy X-ray irradiation. The results are shown in Fig. 6. In this case the main changes occur at lower temperature, as the spectrum is already different after heating at 150 °C for 15 min (not shown). The spectrum immediately after irradiation can be successfully simulated with a significant contribution of CO_3^{3-} radicals, while the spectrum at 175 °C contains only CO_2^-. The changes can thus be explained assuming the formation of CO_3^{3-}, next to CO_2^-, by the irradiation and its fast decay accelerated by the thermal treatment. It is remarkable that by this procedure (irradiation, immediately followed by heating), the aforementioned

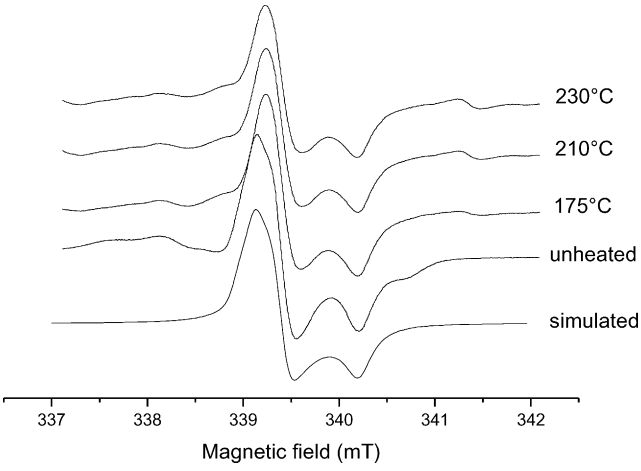

Fig. 6. RT X-band spectra of bone powder immediately after irradiation (X-ray dose 1.5 kGy) as a function of annealing temperature (15 min). P = 0.32 mW, MA = 0.1 mT. The spectrum of the unheated sample is simulated with one CO_2^- and two CO_3^{3-} radicals (EPR parameters given in Table 2).

strong increase of the g = 2.0050 signal is not observed. This could point to transformations between radicals, although it is not simple to propose a reasonable mechanism.

For the Q-band analysis, again a sample was used that had been irradiated several weeks before. The heatings were performed during 30 min at each temperature outside the EPR cavity, since no high temperature cavity for Q-band was available (Fig. 7). The intensity of the signals connected with CO_2^- (g = 2.0019 and 1.9972) decrease while the intensity around g = 2.0047 increases. These experiments with higher resolution seem to indicate that the g = 2.0050 signal observed in X-band (Fig. 5) is most likely the same and thus its g-value should be somewhat corrected downwards. Due to the overlap with the CO_2^- signals, it is clear that a precise g-value determination is not simple. It can even be expected that the real g-value is somewhat smaller than 2.0047 or that the signal is axial or orthorhombic. After heating at 300 °C, there is a very dominant EPR signal at g = 2.0029, probably due to the well-known coal radical [1]. Although not shown in Figs. 5 and 6, the same radical was found in X-band. In view of the weak shoulder on the left in Fig. 7, it cannot be excluded that the g = 2.0047 signal is also still present.

The identification of the radical responsible for the latter signal, is not obvious, as usual when no hyperfine information is available. The above experiments seem to prove that the g = 2.0042 and 2.0060 signals are not related to the g = 2.0047 signal. Next to the CO^- and CO_3^- options, suggested above, also organic radicals could be considered, but it then has to be assumed that there are several radicals involved with g-values in the range 2.0040–2.0060. From a literature study, also no real conclusions can be made in view of the large dispersity in types of experiments and reported g-values, as sketched hereafter. Collagen appears to be rather radiosensitive, but the organic signals exhibit fading [33,34]. Irradiation of collagen normally produces a single broad slightly asymmetric line with a g-value close to 2.004 [35]. An organic

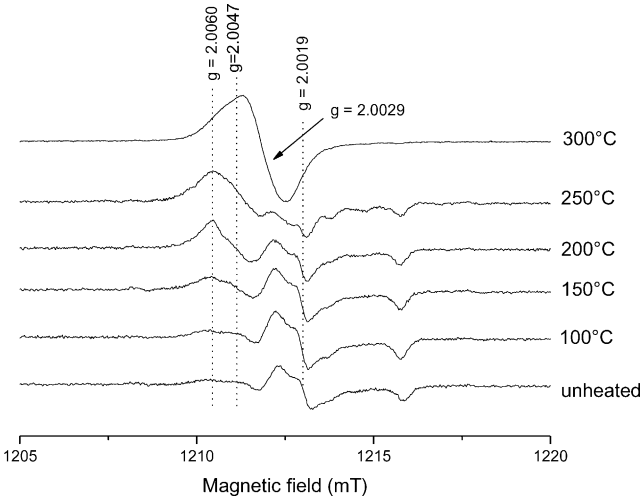

Fig. 7. RT Q-band spectra of irradiated bone powder (^{60}Co-dose 1.4 kGy, 1 month after irradiation) after 30 min annealings at different temperatures. P = 1 mW, MA = 0.2 mT.

signal at $g = 2.0035$, which decayed in 3 days to a plateau value, was reported in Holocene bone [36]. Fading of EPR signals (i.a. CO_3^-) in irradiated chicken legs are described in Onori et al. [37]. Thermal annealing at 200 °C was claimed to destroy the broad organic native signal in cortical femoral bone [26]. In excised human bone, on the contrary, thermal annealing is said to increase collagen signals [10]. From this non-exhaustive review and from our results, it appears that an extensive study of the heating effect on the magnetic resonance signals, as e.g. performed by Brik et al. [7], seems necessary to make progress in the understanding of these complex spectra.

Having identified the major part of the radiation-induced radicals in bone, we now try to make some conclusions about the radical precursors and about the differences between bone and enamel. At variance with enamel, we found no evidence for CO_2^- or CO_2^- precursors in the bulk of the apatite crystallites. As already stated above, the latter are either CO_2 or R–COOH groups located in the intercrystallite phase. Formation mechanisms can be [7]:

$$X, \gamma \rightarrow e^- + h^+ \text{(electron–hole pair formation)}$$

$$CO_2 + e^- \rightarrow CO_2^-$$

$$R\text{–}COOH + X, \gamma, UV \rightarrow R\text{–}CO_2^- + H^+$$

The next important radicals encountered in this study are CO_3^{3-} radicals, quite likely located in the bulk, e.g. on a phosphate site (Z1). Their precursors are probably CO_3^{2-} ions which trap electrons generated by the irradiation:

$$CO_3^{2-} + e^- \rightarrow CO_3^{3-}$$

As for enamel, the origin of the native signal remains quite obscure but probably organic. Finally the detection of isotropic, tumbling CO_2^- radicals ($g = 2.0007$) points to the presence of occluded water in the bone structure.

4. Conclusions

The present EPR study has shown that the radicals formed in irradiated bone are to a large extent the same as in tooth enamel. Again there may be "chaotic" and "fixed" CO_2^- radicals but it cannot be excluded from our experiments that all CO_2^- radicals are "chaotic". The CO_2^- radicals decay after heating around 300 °C. The preheating and the elimination of a major part of the CO_2^- radicals also favors the detection of two types of CO_3^{3-} radicals. Bone and tooth enamel seem to differ with respect to a third type of CO_3^{3-}, which was only found in enamel. Probably related to the more important organic phase in bone, another major difference between bone and tooth enamel seems to be situated in the non-CO_2^- and non-CO_3^{3-} signals. Like for tooth enamel, the elucidation of the nature of the so-called native signals remains a real challenge.

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