Thermal behavior of orientated and non-orientated CO$_2$ radicals in tooth enamel

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ABSTRACT

Isothermal heating experiments on enamel fragments show that there are two pronounced increases in the intensity of the orientated CO$_2$ radicals, after heating for 15 min at 225 °C and 1440 min at 175 °C. While the powder spectra virtually remained unchanged at these heating steps, it was found that the angular intensity variability in the fragments greatly increased. In general, it is not possible to explain these increases by transfers from non-orientated CO$_2$ radicals to orientated, because there are simply not enough non-orientated CO$_2$ radicals in the unheated sample. Instead we attribute the increased angular intensity variability by a higher crystallinity in certain domains in the enamel. It was found that after heating for 15 min at 225 °C, newly occurring orientated CO$_2$ radicals had a somewhat different orientation to the unheated.

1. Introduction

Non-destructive ESR analysis is carried out on enamel fragments instead of powders to minimize the impact of analysis on valuable archaeological samples. However, the ESR spectra of fragments have a high angular dependency which complicates their study and the establishment of experimental protocols. Previous investigations have shown that the angular irradiation response of fragments is different to that of powders due to the presence of two types of CO$_2$ radicals (orientated and non-orientated). The non-orientated (N-O) CO$_2$ radicals give rise to a powder spectrum of the same intensity at all angles. In contrast, the contribution of the orientated radical is changing at each angle, depending on the effective orientation in the enamel fragment (for more details see Grün et al., 2008). The growth of hydroxyapatite crystals in the enamel is complex, forming rhombic prisms with vertical sides, which combine in packages that have complex three-dimensional structures (Macho et al., 2003). Because of this complex structure, an enamel fragment does not have the same properties as a single crystal, i.e. it is not possible to exactly identify the main crystal axes. Although the c-axis is more or less perpendicular to the dentine/enamel junction, it is likely that some contributions of $g_2$ exist when the general prism growth direction is orientated perpendicular to the external magnetic field.

When $g_1$ of the orientated radicals is largest (T1–B1 in Fig. 2), the $g_2$ position (B2 in Fig. 2) is dominated by the N-O radical. Therefore, at this specific position it is possible to extract the component of the orientated CO$_2$ radicals, by fitting the B2 component of a powder spectrum into the B2 of the angular

Fig. 1. Measurement configurations.
Fig. 2. Stacked ESR spectra for X (A, C, E) and Z-configuration (B, D, F) for the unheated (A, B), 15 min at 225 °C (C, D) and 1440 min at 175 °C (E, F). The spectra of the Y-configuration are very similar to the X-configuration.
spectrum and subtracting this from the overall spectrum. However, because of the misalignment of the crystals any ESR intensity in the B2 position will also contain some contribution of the orientated radicals. Unless indicated otherwise, our observations were carried out at this specific angle (150° in X-configuration). One has to keep in mind that the T1–B1 peak may consist of a range of radicals (methyl, CO$_3^-$, CO$_3^-$, etc., for a compilation see Callens et al., 1998; Vanhaelewyn et al., 2000), while any ESR intensity in the B2 is only generated by CO$_2^-$ radicals. It has been speculated, however, that tooth enamel may contain up to seven different types of CO$_2^-$ radicals (Brik et al., 2000b).

2. Material and methods

The experiments were carried out on tooth enamel of a fossil bovid from the archaeological site of Holon (Porat et al., 1999). This sample was already used in the study of Grün et al. (2008). A long lamella was separated from the tooth using a diamond saw, four small fragments removed (62.1, 51.2, 39.5 and 38.0 mg) and the rest of the lamella crushed into powder and divided into 24 aliquots. The fragments were used for isothermal cumulative treatment for 15, 45, 180, 1440 and 14,400 min at 175, 225, 250 and 300°C, respectively. Powder aliquots were heated only once, for 15, 45, 180 and 1440 min at 50, 100, 125, 175, 225, 300°C. The enamel fragments were mounted in three Teflon holders containing a Parafilm mold and were incrementally measured by rotating them around their three major axes. In the context of this paper we used the following configurations: X: rotation around the axis perpendicular to the dentine–enamel junction, Y: around the axis of tooth growth and Z: perpendicular to X and Y (Fig. 1). The sample holders were inserted in a Bruker ER 218PG1 programmable goniometer and measured with a Bruker Elexys E500 ESR spectrometer in 10° increments over 360° with the following measurement conditions: 2 mW microwave power, 0.1 mT modulation amplitude 12 mT sweep width with a sweep time of 21 s. The spectra were accumulated from 50 consecutive measurements. All spectrum

Fig. 3. Heating response of powder (A), merged spectra (B), and position where T1–B1 is maximum (C) and B2 minimum (D).
Fig. 4. Response to heating for 15 min at 225°C. A–I: B2 peak. Angular variations normalised on the maximum intensity unheated, heated and residual (A–C), unheated and heated in polar projection (D–F) and fitted angular intensity variations (G–I). J–R: T1–B1 peak. Angular variations normalised on the maximum intensity unheated, heated and residual (J–L), unheated and heated in polar projection (M–O) and fitted angular intensity variations (P–R).
3. Results and discussion

Fig. 2 shows the stacked spectra of the X and Z-configurations (the stack of the Y-configuration is very similar to X) for the natural sample and two selected heating steps where the largest intensity increases were observed (see Fig. 3). 15 min at 225 °C and 1440 min at 175 °C. In powders, it is thought that the main signal (the peak T1 to dip B1, see Fig. 2A) is dominated by radicals. The T1–B1 peak in powders (Fig. 3A) shows little response to heating up to 225 °C. Only after 1440 min, some of the signal is annihilated. At higher temperatures the signal rapidly decays. The merged ESR spectra of the fragments (the spectra of all angles of the three configurations are aligned and combined, see Grün et al., 2008), behave very similar to the powder (Fig. 3B).

The thermal response of the fragment is very different when specific angles are evaluated. After heating for 180 min, the N-O CO2 radicals show significant annealing at 225 °C and have vanished by 250 °C (Fig. 3D). In contrast, the orientated CO2 radicals begin to decrease at 225 °C and significant intensities can still be measured at 300 °C (Fig. 3C). Between 175 °C and 225 °C, the disappearance of the N-O CO2 radicals seem to be related with a strong increases of the orientated CO2 radicals. After isochronal heating for 15 min, the T1–B1 maximum position (orientated CO2, Fig. 3C) shows an increase at around 225 °C which seems associated with a decrease in the B2 minimum position (non-orientated CO2, Fig. 3D). At longer heating times, there is a second peak occurring 175 °C, which is particularly pronounced in the 1440 min set. This increase is also associated with decreases in B2. It has been speculated by various authors that increases in the orientated CO2 radicals were due to a transfer of N-O CO2 radicals. Brik et al. (1998, 2000a) postulated that a mass transfer between different physical domains of the enamel was responsible while Vorona et al. (2005) speculated that the removal of water molecules was involved.

The question is whether the transfer of non-orientated CO2 radicals into orientated is quantitative and whether the newly created orientated CO2 radicals are the same as those in the natural sample.

Fig. 4 summarises the thermal response of the T1–B1 and B2 peaks after heating for 15 min at 225 °C for all configurations. The B2 peak, which is only generated by CO2 radicals, increases in its maximum and decreases in its minimum (Fig. 4A–F). The diagrams in Fig. 4 are normalised to the maximum intensity in the natural sample. It can be seen in Fig. 4A that there is an angular shift between the natural sample and the heated. This angular offset is particularly pronounced in the X-configuration where we observe a shift by −26° (average determined from the two maxima and minima, in Y and Z-configurations it is 7° and −5°, respectively). The error in these determinations is around 5–10°. We can deduce from this behaviour that the newly created orientated CO2 radicals have a different orientation in the enamel (Fig. 5). The angular intensity variations in the heated sample are the 96, 74, and 57% higher than in the natural in X, Y, and Z-configuration, respectively (Fig. 4G–I). A question arises why these increases are different for the different rotational planes? While the relative differences between the Y and Z-configurations could be attributed to the angular shift by about 26° in the X plane, it is difficult to explain why the angular intensity variation changes strongest in the X-configuration, which is where we actually observe the strongest angular change. In addition, it is not possible to explain the relative increases exclusively by annihilation of N-O CO2 radicals, because their maximum concentration cannot be higher than the ratio of the minimum over maximum B2 in the natural sample, 43% (see e.g. Grün et al., 2008). However, after heating the minimum B2 remained at 36%, which means that no more than 7% of the natural CO2 radicals could have possibly been non-orientated CO2 decaying. One way for explaining the increase in intensity variation is that the newly generated orientated CO2 radicals occur in domains with higher crystallinity, either by moving into different domains, or by crystallisation of certain domains. Perhaps these more crystallised domains have a different orientation. After observing these dramatic changes in the fragment, the most surprising fact is that the power and merged spectra after heating for 15 min at 225 °C are virtually the same, indicating that the total concentration of CO2 radicals did not change at all. At this stage it is not known which specific crystal domains change in crystallinity. We prefer not to resort to speculation and expect that XRD measurements will give further insights.

When evaluating the T1–B1 peak, we observe an angular offset in the newly generated T1–B1 intensity of −30°, 7° and −10° in X, Y, and Z-configuration, which is quite similar to the B2 peak (Fig. 4J–O). The intensity variations of the heated sample are 95, 85, and 102% larger than in the natural in X, Y, and Z-configuration, respectively (Fig. 4P–R). In contrast to the B2 peak, some of the differences in the intensity variations could be explained by the angular shift, which should have the strongest effect on the
Fig. 6. Response to heating for 1440 min at 175 °C. A–I: B2 peak. Angular variations normalised on the maximum intensity unheated, heated and residual (A–C), unheated and heated in polar projection (D–F) and fitted angular intensity variations (G–I). J–R: T1–B1 peak. Angular variations normalised on the maximum intensity unheated, heated and residual (J–L), unheated and heated in polar projection (M–O) and fitted angular intensity variations (P–R).
Z-configuration and the least for Y. The relatively stronger increases in the T1–B1 peak (compared to B2) could be due to the fact that the T1–B1 position is interfered with by a range of other radicals, particularly those which are known to increase by heating, such as SO2, or CO3\(^2\)- (Callens et al., 1995). Nevertheless, changes in the crystallinity are required to explain the increase in the angular intensity variations on the one hand, but the virtually overall unchanged powder spectra, on the other.

Carrying out the same approach for the B2 response to heating for 1440 min at 175 °C (Fig. 6), the angular shifts are 7°, 14° and −7° and the angular intensity variation increases through heating by 65, 35, and 77% in X, Y, and Z-configuration, respectively. For T1–B1 the angular shifts are 10°, 10° and −5° and the angular intensity variation increases through heating by 122, 63, and 99% in X, Y, and Z-configuration, respectively. Because of the errors involved in the assessment in the angular shifts it is not possible to unequivocally state that the newly created orientated CO2 radicals have a different orientation to the natural. The changes in the angular intensity variations of B2 and T1–B1 are very similar to those at 225 °C, above. No more than 8% of the natural CO2 radicals could have been non-orientated which decayed during the heating process. As the powder and merged spectra are again virtually unchanged, changes in the crystallinity are most likely responsible for the intensity variation changes.

4. Conclusions

In contrast to the finding of Brik et al. (2000a), we cannot explain the changes during heating by a transfer from non-orientated CO2 radicals to orientated, because there are simply not enough non-orientated CO2 radicals present in the natural sample to explain the changes in the apparent increase in orientated CO2 radicals. We find that after heating for 15 min at 225 °C some of the non-orientated CO2 radicals have a different orientation in the X-configuration. We could not verify this effect during the heating for 1440 min at 175 °C. The increases in the angular intensity variations can generally be attributed to increased crystallinity, without being able to explain the details and differences in the responses of the B2 and T1–B1 peaks.

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