EPR STUDY OF TOOTH ENAMEL: TEMPERATURE EFFECTS ON CO$_2^-$ RADICALS

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Abstract Thermal treatment effects on the spectral and orientational properties of carbonate radicals created in human tooth enamel by $\gamma$-rays or ultraviolet light are studied using electron paramagnetic resonance.

INTRODUCTION

Stable carbonate radicals (CO$_2^-$) created in tooth enamel after irradiation are used in electron paramagnetic resonance (EPR) dosimetry and dating. Their precursors, CO$_3^{2-}$ molecules can substitute phosphate (B-type) or hydroxyl groups (A-type) in the bulk of hydroxyapatite (Hap). Radicals are supposed to be also on the surfaces of Hap microcrystallites covered with a layer of occluded water and organic impurities. Usually, various radicals are present in irradiated specimens, but their relative amounts depend on the prehistory of the sample. $^{12}$CO$_2$ can be replaced to $^{13}$CO$_2$ in synthetic Hap from the gaseous atmosphere. We used reirradiation of samples after thermal treatments and look for changes in the creation efficiency of various radicals, their thermal stability and reorientation.

EXPERIMENTAL PROCEDURE

Human tooth enamel was crushed in agate mortar, separated from dentine in thallium malonate formate solution with water ($\rho = 2.5$), washed in distilled water and dried at room temperature (RT) over activated charcoal for several days. Carbonate
radicals were created either with $^{60}\text{Co} \gamma$-irradiation (doses 0.5 to 10 kGy in various samples) or ultraviolet light from a KrF laser (248 nm, typical flux on the layer of grains 500 J/cm$^2$). EPR spectra were measured at RT on the ERS 230 spectrometer, the signal intensity was calibrated against a MgO:Cr specimen and the magnetic field scale was converted to g-factors using fine structure lines of ZnS:Mn or the single line of a grain of additively colored CaO ($g = 2.0001(3)$) measured together with enamel probe. All spectra were measured at RT. Samples were heated in the spectrometer sample holder (open quartz vial) in air using a massive Al block with two holes, one for the vial and the other for thermometer. A 16 mg piece of enamel was used for orientation dependence measurements where it was rotated around the axis perpendicular to the magnetic field.

First derivative EPR powder spectra were integrated and fitted using the Kneubühl's theory for CO$_2^-$ radicals with rhombic symmetry and single line for isotropic CO$_2^-$ centers, all convoluted with appropriate Lorentzian or Gaussian using MICROCAL ORIGIN 5.0. The rest could be approximated with one or several Gaussians.

RESULTS AND DISCUSSION

An approximate evaluation of the CO$_2^-$ formation yield of $^{60}\text{Co} \gamma$ rays in the crushed human tooth enamel was made by comparison of integrated EPR spectra of the irradiated specimen and a MgO:Cr calibration standard. The result was $6.5(\pm1)\cdot10^{10}$ spin/(mg-Gy), less than $10^2$ electron volts per radical. The number of thermally unstable CO$_3^-$ and other radicals was less by an order of magnitude at least. An illustration of the EPR spectrum representing its components is given in Figure 1. This spectrum is taken from a temperature series after the final heating at 285 °C of a 45 mg powder specimen which was irradiated with UV light (450 J of the $\lambda=248$ nm light of an KrF laser). The unstable radicals were destroyed and the spectrum was quite simple. The number of CO$_2^-$ radicals was decayed to 50 % of that before heating. The remaining spectrum consists of a composite band of rhombic centers (M), two weak isotropic bands (P, O) and a residual band (N) which may consist the contribution of various other radicals.
Table 1 shows the parameters of the components: g-factors, half-widths Δ, profiles and normalized to unity areas under the contours. Notation of bands according to Moens et al. are given in parentheses. The isotropic band at g = 2.0057 is quite narrow; it grows up from a wider band after heating the sample above 235 °C. Its position did not alter by rotation of a well oriented piece of enamel. This band was not described in papers 2,3,5 but was noticed in 6 as C band.

Reirradiation of the enamel after heating at 285 °C was not less effective than in the case of a virgin sample. In the temperature region above 300 °C the number of created

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**FIGURE 1.** EPR spectrum of irradiated fine grained tooth enamel after quenching at 285 °C (B). Microwave power was 14 mW. Points – integrated experimental spectrum; dotted curve (K) – sum of components (M, N, O, P) of the theoretical approximation.

**TABLE 1.** Parameters of component bands from Figure 1.

<table>
<thead>
<tr>
<th>Notation</th>
<th>g₁</th>
<th>g₂</th>
<th>g₃</th>
<th>Δ, Gauss</th>
<th>Profile</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>M (A3)</td>
<td>2.0031(2)</td>
<td>2.0023(5)</td>
<td>1.9975</td>
<td>1.48</td>
<td>Lorentz</td>
<td>0.89</td>
</tr>
<tr>
<td>O (B)</td>
<td>2.008(0)</td>
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<td></td>
<td>1.2</td>
<td>Lorentz</td>
<td>0.007</td>
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<tr>
<td>P</td>
<td>2.0056(8)</td>
<td></td>
<td></td>
<td>0.4</td>
<td>Lorentz</td>
<td>0.015</td>
</tr>
<tr>
<td>N</td>
<td>2.0024(6)</td>
<td></td>
<td></td>
<td>2.8</td>
<td>Gauss</td>
<td>0.088</td>
</tr>
</tbody>
</table>
radicals decayed with an activation energy $E_D = 1.3$ eV. When the sample was then heated at 415 °C for 10 min, about 1 % of carbonate radicals persisted. After repeated irradiation we could detect rhombic CO$_2^-$ signal but the half width of the band was increased to 3.8 G and the concentration of radicals (creation effectivity) was only about 10 % of that of the new specimen after similar irradiation.

The orientational dependence of EPR spectra of tooth enamel has been detected in several works. In some specimens this dependence is anomalous, because spectral bands change their intensity with rotation angle and not position. In Figure 2 we present angle dependent spectra for a well oriented piece of enamel which has been irradiated with a γ-ray dose 0.5 kGy (showed no anisotropy after that), heated to 200 °C (became anisotropic), reirradiated 5 years later with a dose 6.2 kGy (the spectrum was again nearly powder like) and finally heated in some steps up to 270 °C (anisotropy was build up with an activation energy $E_R = 0.8$ eV). The signal of CO$_2^-$ molecules oriented parallel to the field (at 3430 G) behaves in a complex way: it diminishes at small angle variations from 0° and then displaces additionally toward lower field values. The composed signal from two perpendicular orientations (3420 and 3422 G) decreases from 90° to 0° curves with little apparent shift. The isotropic spectral lines are at 3416.5 and 3425 G (the small shift from Figure 1 is due to different klystron frequencies).

An inspection of the components of fitted spectra shows that the peculiarities of the orientational dependence are mostly due to the nonideal orientation of enamel microcrystals (dimensions of the order of 10$^3$ nm) over the millimeter sized grain. At small rotation angles there are many microcrystallites having perpendicular (or parallel) orientations in respect to the field which contribute to the low field (resp. high field) ends of the first derivative EPR spectra.

Human tooth enamel undergoes metamorphic changes during ageing. One effect is expressed in changes of the single grain spectrum, which develops anisotropy when heated. Reirradiation studies lead us to the conclusion, that those changes are not explained with the rearrangement of microcrystals. Additional spectrum was of the powder type and it became of the oriented crystal type after second heating. A possible explanation is
that radical molecule ions reorient themselves thus increasing the number of molecules with symmetry axis parallel to the crystal c axis. Reorientation of molecules can also occur with jumps into lattice positions with higher symmetry, for instance into A sites (hydroxyl vacancies).

SUMMARY

CO$_2^-$ carbonate radicals created in tooth enamel with γ-irradiation or ultraviolet light rearrange at temperatures around 250 °C ($E_R = 0.8$ eV) and decay above 300 °C ($E_D = 1.3$ eV).
ACKNOWLEDGEMENT

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REFERENCES

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