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X-Band Rapid-Scan Electron Paramagnetic Resonance of Radiation-Induced Defects in Tooth Enamel

Zhelin Yu^a, Alexander Romanyukha^b, Sandra S. Eaton^a, and Gareth R. Eaton^{a,1}

^aDepartment of Chemistry and Biochemistry, University of Denver, Denver, Colorado 80208

^bNaval Dosimetry Center, Bethesda, Maryland 20889

Abstract

X-band rapid-scan electron paramagnetic resonance (EPR) spectra from tooth enamel samples irradiated with doses of 0.5, 1 and 10 Gy had substantially improved signal-to-noise relative to conventional continuous wave EPR. The radiation-induced signal in 60 mg of a tooth enamel sample irradiated with a 0.5 Gy dose was readily characterized in spectra recorded with 34 min data acquisition times. The coefficient of variance of the calculated dose for a 1 Gy irradiated sample, based on simulation of the first-derivative spectra for three replicates as the sum of native and radiation-induced signals, was 3.9% for continuous wave and 0.4% for rapid scan.

INTRODUCTION

To properly handle an emergency medical response or to perform radiation epidemiological studies long after a radiological event has occurred, it is critically important to determine the radiological doses that were received by individuals. Since most people do not wear radiation dosimeters, their radiation doses can be measured only by biodosimetry techniques. An overview of applicability under different scenarios has been shown in reports by Simon *et al.* and Alexander *et al.* (1, 2). The generation of free radicals is a primary effect of radiation exposure that occurs immediately upon exposure to radiation. Electron paramagnetic resonance (EPR) is one of a very few techniques capable of directly measuring radiation-induced free radicals, and the EPR signal is not affected by stress, wounds or medical treatment. These advantages make EPR unique from other biodosimetry techniques that require significant time after a radiation-induced casualty event to become applicable. Two types of tissue are known to be suitable for EPR dosimetry: calcified tissue (teeth and bones) and keratin-based tissue (nails and hair). Tooth enamel has the best EPR dosimetric properties because of the high stability of the radiation-induced radicals. Radiation exposure produces a characteristic long-lasting EPR signal in tooth enamel that has different *g* values and electron spin relaxation times than the native signal (3). This signal is the basis for retrospective accident dosimetry based on teeth (3).

During the past decade, EPR dosimetry in teeth has made considerable progress towards becoming a routine dosimetric method for radiation epidemiological studies. An

¹Address for correspondence: Department of Chemistry and Biochemistry, University of Denver, 2101 E. Wesley Ave., Denver, CO 80210; geaton@du.edu.

interlaboratory comparison indicated that there are two challenges to the accuracy of dosimetry: improvement of the signal-to-noise (S/N) and reproducibility of sample positioning (4). The focus of the current study was on improving S/N .

Rapid-scan EPR has been developed as a method to improve S/N relative to conventional continuous wave (CW) experiments. In rapid-scan EPR the magnetic field is scanned through the signal in times that are short relative to the electron spin relaxation times (5). The directly detected quadrature signal is obtained using a double-balanced mixer with the reference at the resonance frequency. By contrast conventional CW EPR uses phase-sensitive detection at the modulation frequency (5). Deconvolution of the rapid-scan signal gives the absorption spectrum. Rapid-scan EPR has been shown to yield improved S/N per unit time relative to CW EPR for rapidly tumbling nitroxides in fluid solution (6), immobilized nitroxides (7), spin-trapped superoxide (8), the E' center in irradiated fused quartz (9), amorphous hydrogenated silicon (10), $N@C_{60}$ diluted in C_{60} (10) and the neutral single substitutional nitrogen centers (N_S^0) in diamond (10). The improved S/N for rapid-scan relative to CW spectra comes from three factors: 1. Differences in signal amplitudes due to excitation of a small portion of the spectrum in the CW experiment versus excitation of the entire spectrum in rapid scan; 2. The ability to use higher B_1 without power saturating the signal; and 3. The differences in the noise spectral densities in CW and rapid-scan spectra (5).

In this article we show the improvement in S/N that can be obtained by employing rapid scan to detect the radiation-induced signal in tooth enamel at X band.

METHODS

Sample Preparation

Tooth enamel samples were prepared from six human molars collected from the U.S. population (extracted for medical reasons and collected under conditions of anonymity). Tooth enamel was separated from dentin using a low-speed water-cooled dental drill. The pooled tooth enamel from all samples was then washed with distilled water and dried at 40°C under vacuum for 14 h. The enamel pieces were then crushed with a mortar and pestle and carefully mixed. The tooth enamel powder with grain size of 0.12–0.50 mm was segregated for the measurements using sieves. The pooled sample obtained by this procedure was split into portions and 0.5 and 10 Gy irradiated. Tooth enamel samples were irradiated using a calibrated ^{137}Cs source with air kerma dose rate of 1 Gy/min. Tooth enamel samples (60 mg) at each irradiation level were placed in 4 mm outer diameter quartz EPR tubes. A 60 mg sample of tooth enamel powder with smaller grain size (<0.12 mm) was placed in an EPR tube to record the “native” (0 Gy dose) EPR signal. The other sample of tooth enamel with grain size <0.12 mm was used for 1 Gy irradiation.

Sample Irradiation

Tooth enamel samples were exposed at a dose rate of approximately 1 Gy/min using a ^{137}Cs irradiator (Gammacell® 40; Atomic Energy of Canada Ltd., Mississauga, Canada). The irradiator dose rate was verified using a U.S. Navy thermo luminescence dosimeter (TLD) as

described by Reyes (11). The U.S. Navy dosimetry system DT-702/PD is traceable to the NIST ^{137}Cs source and is in full compliance with the requirements of the American National Standard for dosimetry ANSI HPS N13.11-2009. Detailed descriptions of DT-702/PD can be found in previously published reports (12–14). Reyes estimated that the bias between the Navy TLD dosimeters and the ^{137}Cs irradiator was 6% (11).

EPR Spectroscopy

A Bruker E500T spectrometer (Bruker Inc., Billerica, MA) was used to record CW and rapid-scan spectra with a Bruker Flexline ER4118X-MD5 dielectric resonator, which can excite spins over a sample height of 1 cm. Since sample heights were about 6 mm, uniform B_1 and scan field were ensured along the full length of the samples. The Q of the resonator is ~9,000 for these nonlossy samples. A Bruker SpecJet II fast digitizer was used to record rapid-scan spectra. The phase difference among the quadrature detection channels was calibrated with a small sample of solid BDPA (1:1 α,γ -bis(diphenylene- β -phenylallyl):benzene) radical (7). The deviation between the Kronig-Kramers transformation of the absorption signal and the observed dispersion signal showed that a phase correction of 7° was required. This correction was applied in the post-processing of the rapid-scan signals.

The sinusoidal scans were generated with the recently described scan driver (15). The scan coils were constructed from 200 turns of Litz wire (255 strands of AWG44 wire). The coils have about a 7.6 cm average diameter and were placed about 4 cm apart. The coil constant was 3.77 mT/A, which is sufficient to generate scans up to 6.0 mT wide with scan frequencies up to 35 kHz. Mounting the coils on the magnet, rather than on the resonator, reduces the oscillatory background signal induced by the rapid scans. The placement of highly conducting aluminum plates on the poles of the Bruker 10" magnet reduces resistive losses in the magnet pole faces that arise from induced currents. The dielectric resonator decreases eddy currents induced by the rapidly changing magnetic fields relative to resonators with larger amounts of metal. Data were acquired in blocks of 5–6 sinusoidal cycles. For a sinusoidal scan the rate varies through the spectrum and is $\pi f_s B_m$ T/s at the center for the scan. For the spectra used in this study, the scan rate at the center of the spectrum is 660 T/s, which is $\pi f_s B_m$ T/s, where f_s is the scan frequency (35 kHz) and B_m is the scan width (6.0 mT).

To select the incident microwave powers for the CW and rapid-scan experiments, power saturation curves were examined from the sample irradiated with 10 Gy (Fig. 1). At this dose the EPR spectrum is dominated by the radiation-induced signal that is assigned to CO_2^- (3). The amplitudes of peaks in CW spectra and rapid-scan signals were measured as a function of microwave power. The incident powers were converted to B_1 using the known resonator efficiency of about $380 \mu\text{T/W}^{1/2}$ at Q of 9,000 (20). The power selection was done using the following criterion. A linear least-squares fit through the point 0,0 and the signal amplitudes at the lowest 5 or 6 microwave powers was extrapolated to higher B_1 . The B_1 selected for data acquisition was the point at which the experimental signal amplitude was about 5% lower than the amplitude predicted by linear extrapolation of the non-saturated signal amplitude. The modulation frequency for the CW spectra was 100 kHz. The modulation amplitudes (0.076 mT) were about 20% of B_{pp} of the radiation-induced signal

in tooth enamel. These parameters result in less than 2% line broadening relative to spectra obtained at lower modulation amplitude and smaller B_1 . The data acquisition times were about 34 min. The estimates of 34 min acquisition times were based on the following calculations, which are consistent with the relatively small overhead in the software. For CW spectra the acquisition time is the conversion time per point multiplied by the number of field steps. For rapid scans the acquisition time is $(1/f_s)$ multiplied by the product of number of scan cycles combined in the deconvolution software and the number of scans averaged.

Signal Processing

The rapid-scan signals were deconvolved, background removed and baseline corrected (16, 17). In the deconvolution procedure the signals from the two quadrature channels for up-field and down-field scans are combined (18). A post-processing Gaussian filter was applied to both CW and rapid-scan spectra. The bandwidth of the first derivative spectrum is larger than for the absorption spectrum of the same signal. The first derivative spectrum was therefore calculated by numerical differentiation of the deconvolved rapid-scan spectrum before low-pass filtering, with subsequent application of low-pass filtering. S/N is the peak-to-peak signal amplitude for CW or signal amplitude for rapid scan divided by rms noise in baseline regions of the spectrum.

RESULTS

Comparison of CW and Rapid-Scan Spectra of 10 Gy Irradiated Tooth Enamel

Figure 2 compares CW and rapid-scan spectra of a 10 Gy irradiated tooth enamel sample. The spectra are in good agreement with previously reported studies (3, 19). The peaks observed at about 343.9 and 343.1 mT with a microwave frequency 9.62 GHz correspond to the reported g values of $g_{\perp} = 2.002$ and $g_{\parallel} = 1.997$ for the dose-dependent signal (3, 19). The native signal has longer electron spin relaxation times and saturates more readily than the radiation-induced signal (20, 21). The B_1 selected for the CW and rapid-scan experiments selectively enhances the radiation-induced signal relative to the native signal (20). The weak peak from the native signal observed at about 342.5 mT (Fig. 2) is consistent with those reported in the literature of $g = 2.0045$ and a line width of 0.7–0.8 mT (3). The S/N for the rapid-scan spectrum is substantially higher than for the CW spectrum recorded with the same data acquisition time (Fig. 2).

Radiation-Induced Signals in Tooth Enamel Samples with Lower Radiation Dose

One of the main objectives of this work was to investigate the ability of the rapid-scan technique to improve S/N and thereby improve resolution of radiation-induced and native signals in irradiated tooth enamel samples. Therefore it was important to study samples with different (variable) contributions of these two signals to the EPR spectrum of the tooth enamel samples. To vary the relative intensities of the native and radiation-induced signals, different radiation doses (e.g., 0, 0.5, 1 and 10 Gy) were examined. The relative intensity of the native and radiation-induced signals depends also on the sample grain size (3). That is why the 0 and 1 Gy samples were chosen to have a smaller grain size (<0.12 mm) than the 0.5 and 10 Gy samples (0.12–0.50 mm). Known differences in the grain size for samples that were 0.5, 1 and 10 Gy irradiated can be responsible for up to 60% difference in

radiation sensitivity for samples irradiated with 1 Gy compared to 0.5 and 10 Gy. Fattibene and Callens (3) described several effects of grain size on the EPR signals in tooth enamel: 1. The dose conversion may depend on grain size; 2. Defect stability may depend on grain size; 3. The surface may be a larger factor for smaller grain size; and 4. Samples with different grain size may result in differences in the EPR resonator filling factor.

Spectra of tooth enamel irradiated with 1 Gy and a native sample are shown in Fig. 3. For the native sample or tooth enamel samples irradiated with 0.5 or 1 Gy the conventional CW spectra obtained with 34 min data acquisition time were extremely noisy. To acquire data with adequate S/N for analysis would have required very long signal averaging time. Thus, only the rapid-scan spectra are shown in Figs. 3 and 4. The smaller grain size in the native (0 Gy) sample increases the intensity/gram of the native signal so 60% of the amplitude of the native signal was subtracted from the spectra of samples irradiated with doses of 1.0 or 0.5 Gy. The resulting difference spectrum for the 1 Gy sample has the line shape expected for the dose-dependent signal, which is seen more clearly in the first derivative than in the absorption spectrum. The S/N is good enough to estimate the dose. The peak-to-peak amplitude of the first-derivative signal is 16% of that observed for the 10 Gy sample, which indicates a dose of 1.6 Gy. As discussed above, the known difference in the grain size for samples with 0.5, 1 and 10 Gy can be responsible for the difference in radiation sensitivity for samples irradiated with 1 Gy compared to those irradiated with 0.5 and 10 Gy.

The spectrum for the 0.5 Gy irradiated sample and the result obtained by subtraction of the native signal are shown in Fig. 4. Although the S/N for the difference spectrum is low, the features due to the dose-dependent signal are still clearly discernible with a peak-to-peak amplitude of the first-derivative spectrum about 5% of that for the 10 Gy sample, which is in good agreement with the reported dose of 0.5 Gy.

For the 1 Gy sample three replicate EPR spectra were recorded by CW and three by rapid scan (Supplementary Table S3; <http://dx.doi.org/10.1667/RR14032.1.S1>). The coefficient of variance of the calculated dose based on simulation of the first-derivative spectra as the sum of native and radiation-induced signals was 3.9% for CW and 0.4% for rapid scan. Thus, the improved S/N results in substantially improved precision in the calculated dose.

DISCUSSION

For the same data acquisition time and conservative data acquisition parameters the S/N for rapid-scan EPR of irradiated tooth samples is substantially higher than for conventional CW spectra. The X-band rapid-scan spectra provide S/N for irradiated tooth enamel samples that are sufficient to clearly characterize the dose-dependent signal at a dose of 0.5 Gy. The S/N of each method could be increased by using less conservative parameters. For example, one could allow somewhat more line shape distortion and use higher modulation amplitude in the CW spectra. (Supplementary Table S3; <http://dx.doi.org/10.1667/RR14032.1.S1>). Up to a modulation amplitude of about half the line width, S/N will increase nearly linearly with increased modulation amplitude. Similarly, higher microwave power could be used for both CW and rapid-scan spectra. The 5% deviation from linearity was an arbitrary criterion, to ensure accurate line shapes that aid in separating the two overlapping signals. Rapid scan

could be obtained at a higher scan rate, which would allow higher power without increase in saturation, but at some point the resonator bandwidth would start to over-filter the signal and distort the line shape (5). Higher resonator Q could be used, but for rapid scan the filter effect of the Q could be a problem, as just described, and in CW EPR higher Q could result in higher noise (22).

The difference spectrum for the 0.5 Gy sample (Fig. 4) was based on a single spectrum for the native signal. With replicate measurements for the native sample it should be possible to characterize radiation doses significantly lower than 0.5 Gy.

Although EPR in tooth enamel has been successfully applied in many radiation epidemiological studies (3), it is difficult to apply in case of emergency because extracted teeth are not readily available in this situation. Recent instrument developments have made it possible to perform *in vivo* EPR dose measurements in teeth (23), but there is a problem with low S/N for the dose of concern, which is 1–2 Gy (23). The rapid-scan technique could be an approach to improving S/N for *in vivo* EPR dosimetry.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG. 1.

Power saturation curves for the radiation-induced signal in irradiated tooth enamel (10 Gy) obtained by conventional CW ($\diamond$) or rapid scan ($\blacklozenge$). The powers selected for recording the CW and rapid-scan spectra shown in Fig. 2 are indicated with an arrow or circle, respectively.

FIG. 2.

EPR spectra of 60 mg of irradiated tooth enamel (10 Gy) obtained with 34 min acquisition time at 9.62 GHz. Section A: Absorption spectrum obtained by rapid scan with $B_1 = 27.6$ μ T. Section B: First derivative of spectrum in section A. Section C: Conventional CW spectrum obtained with 0.076 mT modulation amplitude and $B_1 = 5.5$ μ T. The microwave B_1 for the two methods was selected to give comparable degrees of power saturation of the radiation-induced signal. The arrows mark the peak due to the native signal.

FIG. 3.

Rapid-scan EPR spectra of 60 mg irradiated tooth enamel obtained with $B_1 = 27.6 \mu\text{T}$ and 34 min acquisition time. Section A: Absorption spectra of 1 Gy irradiated sample (—), native signal (— —), and difference between 1 Gy irradiated spectra and native signal (— · —). Section B: First derivatives of spectra in part A for 1 Gy irradiated sample (—) and the difference spectrum (— —).

FIG. 4.

Rapid-scan EPR spectra of irradiated tooth enamel obtained with $B_1 = 27.6 \mu\text{T}$ and 34 min acquisition time. Section A: Absorption spectra of 0.5 Gy irradiated sample (—), native sample (---) and difference between 0.5 Gy irradiated spectra and native sample (- -). Section B: First derivatives of spectra in section A for 0.5 Gy irradiated sample (—) and the difference spectrum (- -). The arrows highlight features of the radiation-induced signal.