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COMPARATIVE EPR ANALYSIS OF MODERN AND FOSSIL TOOTH ENAMEL: UNVEILING AGING-INDUCED COMPONENTS

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This study involves comparing EPR signals from three-year-old modern cow tooth enamel with the spectra of fossil tooth enamel exposed to natural background radiation over an extended period. The EPR spectrum of the significantly aged fossil tooth enamel displays additional components absent in the EPR spectra of the modern tooth enamel. Specifically, the septet signal associated with isopropyl (or alanine) radicals is not observed in the EPR signals of modern tooth enamel when irradiated up to 1.3 kGy. It is hypothesized that the isopropyl radicals present in fossil tooth enamel are not a result of radiation but rather stem from the natural breakdown of organic components due to the aging process. This characteristic is proposed as a dependable tool for authenticating tooth samples.
Keywords: *Modern tooth enamel; Fossil teeth; EPR dosimetry; Isopropyl radical*
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INTRODUCTION

EPR dosimetry and dating rely on identifying and quantifying an Electron Paramagnetic Resonance (EPR) signal induced by ionizing radiation, provided the signal intensity was once reset to zero. Implicit in this approach is the assumption that a correlation exists between the intensity of the radiation-induced signal and the absorbed radiation dose. This fundamental principle underlies the use of tooth enamel as a natural dosimeter in EPR dosimetry and dating [1]–[5]. Irradiated tooth enamel exhibits a stable EPR signal, the intensity of which corresponds to the absorbed dose [6].

It is well established that the EPR signal in fossilized tooth enamel is of a composite nature, necessitating the isolation of the radiation-induced EPR signal from other paramagnetic signals [4], [7]–[9]. In tooth enamel or bone suitable for dating, a stable CO^{2-} radical generated by radiation is a key signal. Other radicals induced by irradiation, such as CO_3^{3-} and CO^{3-}, are irrelevant for retrospective dosimetry or dating due to their instability [10].

Additionally, the EPR spectrum of tooth enamel contains a native signal present in non-irradiated modern tooth enamel. However, even the youngest tooth is not exempt from weak, radiation-induced EPR signals due to natural background irradiation. Modern enamels from human and other mammals' teeth, when irradiated in the laboratory, have been widely studied using the EPR method. A general observation is that the complete EPR line shape of laboratory-irradiated tooth enamel differs from that of naturally irradiated enamel, although the central part of the spectrum is easily reproducible.

The focus of the current study is a comparison of EPR signals obtained from three-year-old modern cow tooth enamel with the spectra of fossil tooth enamel that has been exposed to natural background radiation for an extended period.

EXPERIMENTAL

The objects under investigation included a remarkably well-preserved fossil tooth from an elephant (Palaeoloxodon antiquus) discovered in the Mingachevir district of Azerbaijan in 2010, as well as a three-year-old modern cow tooth. The extinct straight-tusked elephant (Palaeoloxodon antiquus) once inhabited Europe during the Middle and Late Pleistocene, approximately 781,000 to 50,000 years before the present. Initially believed to be closely related to the living Asian elephant, a shift occurred in 2016 when DNA sequence analysis revealed that its closest living relative is the African forest elephant, Loxodonta cyclotis. Surprisingly, it is more closely related to L. cyclotis than L. cyclotis is to the African bush elephant, L. africana. This finding challenges the current classification of the genus Loxodonta, as outlined in E. Callaway's article "Elephant history rewritten by ancient genomes" in Nature, published in September 2016 (https://doi.org/10.1038/nature.2016.20622). The procedures for sample preparation and Electron Spin Resonance (ESR) measurements were as follows: Initially, the enamel was carefully extracted from the teeth using a dental drill with water cooling. The 1.5-mm average thickness enamel was then immersed in a 30% NaOH solution for a day to disinfect and separate any remaining dentine.

For the fossil tooth enamel samples, a dental drill was employed to remove approximately 50±5 μm from both the inside and outside of the enamel surface, ensuring that natural alpha radiation did not impact the results. In total, 2 g of enamel was collected from both the fossil and modern tooth and air-dried at room temperature for three days. Half of the samples were powdered using an agate mortar, and powder with a size range of 100–50 μm was isolated for subsequent

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measurements. The remaining portion was retained as a single fragment of enamel measuring 4mm×1mm×1.5mm. Both enamel powder (0.1 g) and single fragment (bulk) samples were individually placed inside Suprasil glass tubes for the EPR signal measurements.

The ESR signal of the samples was assessed using a Bruker EMXplus (X-band) spectrometer. The spectrometer was configured with the following parameters: a central field of 3,520 G, a scan range of 100 G, an amplitude modulation of 3 G, a modulation frequency of 100 kHz, a time constant of 20.48 ms, and a power of 2.14 mW unless otherwise specified in the text. Subsequently, the samples underwent irradiation at room temperature utilizing a ^{60}Co source, with additional doses applied, and ESR signals were measured under identical conditions.

The dose rate of the ^{60}Co source was determined using the Magnettech Miniscope MS400 EPR Spectrometer, employing individually wrapped barcode-labeled BioMax Alanine Dosimeter Films (developed by Eastman Kodak Company).

RESULTS AND DISCUSIONS

The EPR signal in fossil tooth enamel manifests as an asymmetric signal characterized by three peaks at g ~ 2.0043 (T1), g ~ 2.0013 (B1), and g ~ 1.9985 (B2) (refer to Fig. 1A (1)). The primary contributor to this signal is identified as the CO^{2-} radical [11], although other radicals, predominantly carbonate-derived radicals and certain oxygen radicals [11], are suggested to play minor roles. Additionally, a signal at position "a" is observed, attributed to the isopropyl radical with a hyperfine splitting of 2.17 mT [1][4][12]. This septet signal has been previously noted in middle Pleistocene tooth samples by other researchers [13]. However, Duval [14] associated this signal with "free diethyl" radicals. While the experimental separation of the central signal is challenging, the system is commonly simplified by considering three main types of CO^2 [11]: one isotropic at g ~ 2.0006 and two anisotropic CO2 radicals—an axial ($g_\perp$ ~ 2.003; $g_\parallel$ ~ 1.997) and an orthorhombic (g_x ~ 2.003; g_y ~ 1.997; g_z ~ 2.001). Owing to differences in thermal stability and microwave saturation characteristics, the relative proportions of these signals in the ESR signal may vary between natural and irradiated spectra, leading to the observation of distinct yet closely situated g values at positions T_1, B_1, and B_2.

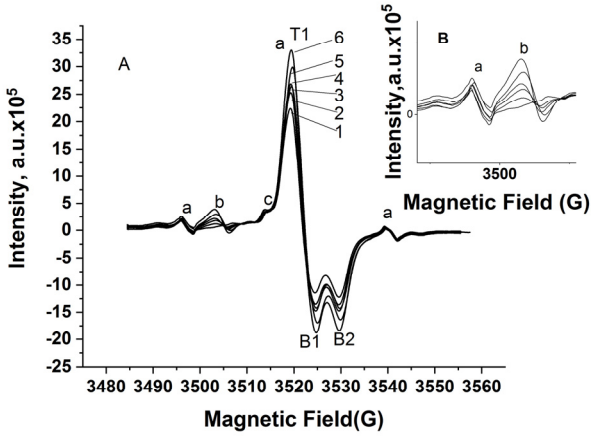

Figure 1. Dose-response spectrum of fossil tooth enamel powder

In panel A, the spectra are presented for various conditions: natural, without additional laboratory dose (1); irradiated at 44.7 Gy (2); 89.4 Gy (3); 114.1 Gy (4); 178.8 Gy (5); and 223.5 Gy (6). The dose rate was 0.149 Gy/s. In panel B, a segment of the spectra around 3,500 G is highlighted. Punctuation and identification of EPR signals have been adopted from [14]: (i) The signal labeled "a" represents a septet centered on the primary CO_2^- signal at g = 2.0043, formed by a free dimethyl radical, with only three lines visible in that magnetic field range; (ii) the isotropic line (marked "b") at g = 2.0114 could be ascribed to CO_3^-; and the isotropic line at g = 2.0075 (marked "c") is typically attributed to a free radical, likely SO_2^- Positions T1, B1, and B2 are indicative of the primary EPR signal

Upon laboratory irradiation, there is an augmentation in the EPR signal, as illustrated in Fig. 1, depicting signal intensity at different doses. Noticeable peak increases occur in the central part of the spectra and at position "b," while peaks associated with isopropyl radicals (position a) remain unchanged (refer to Fig. 2b).

The identical samples were also assessed six months later, as depicted in Figure 2 A minor reduction in the intensity of the central signal was noted, while the signal at position "b" returned to its initial level (refer to Fig. 3B). The intensity of the signal at position "a" remained constant.

The EPR signal in the non-irradiated modern tooth sample is exceedingly faint, requiring special efforts to discern it from the noise signal. Upon subjecting the sample to additional laboratory irradiation, the typical central signal of tooth enamel becomes observable.

The EPR spectra illustrating the dose response of the modern tooth sample are presented in Fig. 3. The tooth samples underwent irradiation with ^{60}Co, ranging from a dose of 174 Gy to 1,305 Gy. The EPR signal of the modern cow tooth enamel is characterized by an asymmetric signal with three peaks at g ~ 2.0044 (T1), g ~ 2.0020 (B1), and g ~ 1.9987 (B2). Notably, the positions of these peaks exhibit minimal changes when compared to the EPR signal of fossil tooth enamel.

A distinctive aspect of the EPR spectra of the modern tooth is the absence of both the peak at position "a" and the peak at position "b." Moreover, these peaks do not appear in the spectrum even up to the irradiation dose of 1.305 Gy. EPR studies on modern tooth samples have been conducted by several researchers [15]–[20], and a general consensus emerges that direct irradiation does not lead to the generation of an EPR signal at position “a”.

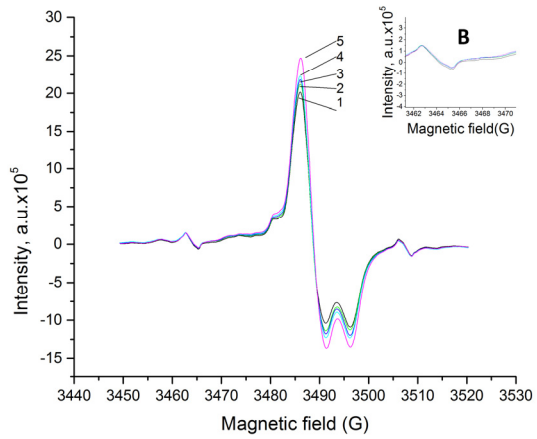

Figure 2. Dose response spectra of fossil tooth enamel powder six months later: irradiated at 44.7 Gy (1); 89.4 Gy (2); 114.1 (3); 178.8 Gy (4); 223.5 Gy (5). B: insert is a magnified part of the spectrum around 3,470 G

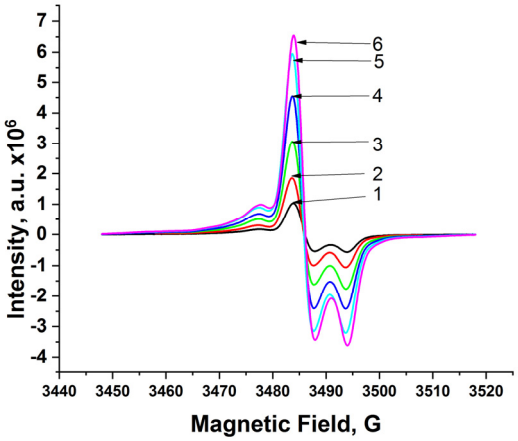

Figure 3. Dose response spectra of modern cow tooth enamel powder: irradiated at 174 Gy (1); 348 Gy (2); 522 Gy (3); 783 Gy (4); 1,044 Gy (5); 1,305 Gy (6)

Ikeya [13] was the first to report the presence of paramagnetic organic radicals at position "a" in γ-irradiated biogenic crystals, such as tooth enamel and fossil shells. The quintet signal with g = 2.0037 and A = 21.9 G was associated with alanine radicals, CH3CH(NH2)COO*, generated from the organic constituent protein in shells (Polinices). He proposed that alanine radicals might be produced by natural irradiation from the alanine amino acids derived from decomposed proteins. According to [20], the intensity of the mentioned signal was not enhanced by further γ-irradiation, and no alanine radicals were detected in fossil shells and bones younger than 10^4 years. In the EPR spectrum of the aragonitic shell (Polinices), which was age-dated using 230Th/234U dating to be 65,000 years old, two additional lines were identified within the quintet signal initially attributed to alanine radicals [13][21]. In this context, the septet signal was associated with isopropyl radicals. The septet signals arising from $(CH3)_2C$--R radicals coincide with a peak of the central line featuring hyperfine splitting of 21.7 G [21]. This septet spectrum has also been observed in fossil horse molars and certain shells, with the radical identified as the isopropyl radical. This radical is known to form in synthetic valine-doped $CaCO_3$ [22].

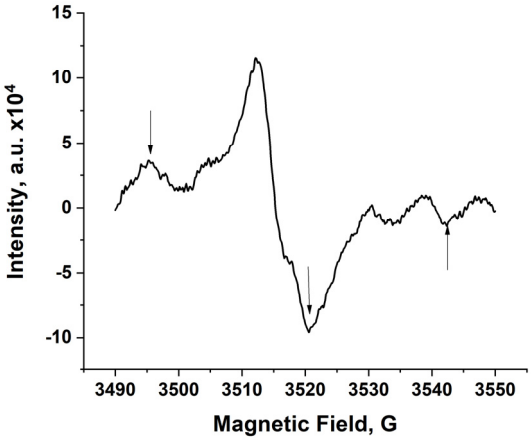

Figure 4. EPR spectrum of the modern cow teeth enamel heated at 160°C for 100 hours. (Central Field 3520 G, Power 2.18 mV, Modulation Frequency 3.2 G, Time constant 20.48 msec, Number of scans 20)

According to annealing experiments conducted with modern tooth enamel from an elephant [23], the alanine (or isopropyl) signal did not manifest solely upon γ-irradiation (400 Gy); it only appeared after subsequent heating, for instance, at 160°C for 48 hours. Furthermore, thermal pretreatment without prior irradiation did not result in the generation of these radicals in recent tooth enamel.

The signal identified at position "a" has been documented in previous studies [22][24], and is acknowledged as indicative of sample annealing due to its presence in the EPR spectra of samples subjected to annealing both before and after irradiation. Notably, the signal at position "a" fails to manifest at 160°C for 48 hours when only the heating stage is applied. However, in our experiments, by prolonging the heating of modern tooth enamel for 100 hours at 160°C, the signal at position

"a" became observable without irradiation, displaying the characteristic quintet signal with g ~ 2.0037 and A ~ 22 G (see Fig. 4).

The peak at position “a” (refer to Fig. 1) is attributed to the presence of isopropyl radicals, indicating that they are not generated during irradiation. Consequently, we assert that the EPR signal observed in fossil tooth enamel at position "a" does not result from radiation but instead stems from the natural decomposition of organic components in tooth enamel due to the aging process.

These characteristic holds potential for various applications and may serve as a quick test to distinguish between ancient and contemporary tooth samples.

CONCLUSIONS

The study involves analyzing EPR spectra of fossil and modern tooth enamel samples, with the fossil sample exhibiting a composite nature requiring careful isolation. In contrast, the modern tooth lacks certain signals, suggesting differences in composition or irradiation effects. The EPR signals were measured using a Bruker EMXplus spectrometer, and subsequent irradiation and dose-response observations were conducted. Six months later, a slight decrease in the central signal's intensity was noted, and the signal at position "b" returned to its original level. Additionally, the absence of peaks at positions "a" and "b" in the modern tooth distinguishes it from the fossil tooth. The presence of isopropyl radicals at position "a" is attributed to sample annealing, unrelated to irradiation, providing potential applications for authentication and differentiating ancient from contemporary tooth samples. The absence of this signal in modern teeth subjected to irradiation may indicate forgery in cases of presenting them as ancient specimens.

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ПОРІВНЯЛЬНИЙ АНАЛІЗ ЕПР СУЧАСНОЇ ТА ВИКОПАНОЇ ЗУБНОЇ ЕМАЛІ: ВИЯВЛЕННЯ КОМПОНЕНТІВ, ІНДУКОВАНИХ СТАРІННЯМ

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Це дослідження передбачає порівняння сигналів ЕПР від зубної емалі сучасної трирічної корови зі спектрами викопної зубної емалі, яка піддавалася впливу природного фонового випромінювання протягом тривалого періоду. Спектр ЕПР викопної зубної емалі значного віку демонструє додаткові компоненти, відсутні в спектрах ЕПР сучасної зубної емалі. Зокрема, септетний сигнал, пов'язаний із ізопропіловими (або аланіновими) радикалами, не спостерігається в сигналах ЕПР сучасної зубної емалі при опроміненні до 1,3 кГр. Гіпотетично ізопропілові радикали, присутні у викопній зубній емалі, не є результатом радіації, а походять від природного розпаду органічних компонентів внаслідок процесу старіння. Ця характеристика пропонується як надійний інструмент для аутентифікації зразків зубів.

Ключові слова: *сучасна зубна емаль; викопні зуби; ЕПР дозиметрія; ізопропіловий радикал*