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ORIGINAL PAPER

EPR study of γ-irradiated feather keratin and human fingernails concerning retrospective dose assessment

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Abstract. In this paper we report the results of comparative EPR studies on free radicals generated in γ-irradiated powder samples of feather keratin powder and human fingernails. In degassed samples of both materials irradiated at 77 K the major component of EPR spectrum represents sulphur-centred radicals in cysteine residues. It quickly decays after air admission at room temperature while a singlet assigned to semiquinone melanin radicals remains the only one seen. The singlet recorded with fingernails decays slowly at room temperature and might be potentially useful for dose assessment by EPR. The advantages and limitations of fingernails EPR dosimetry are discussed.

Key words: EPR spectroscopy • keratin • melanin • radicals • fingernails • dosimetry

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Introduction

The retrospective dosimetry based on the electron paramagnetic resonance (EPR) measurements of paramagnetic centres generated by radiation in teeth and bones is well established [5, 7]. The EPR methodology is suitable for radiation dosimetry due to its accuracy, sensitivity and fast measuring procedure. The EPR bone dosimetry plays currently an important role in personal dose estimation and was successfully used in a few accidents caused by the failures with the operation system of radiation devices [13, 18]. The disadvantage of that method lies in the necessity of tooth extraction or bone biopsy.

The ionizing radiation generates free radicals not only in bones but also in more accessible parts of human body as fingernails or hairs, for example. However, the paramagnetic centres in fingernails and hair are less stable than those detected in bones or teeth. The possibility of using fingernail and hair in natural form for the accidental dosimetry had been tested in a few laboratories [2, 4, 15].

The dosimetry based on the measurement of the intensity of EPR signal generated in fingernails has a potential to be widely used because the samples for measurements can be obtained in an easy way. It was reported earlier that using this method radiation dose could be estimated in the range from a few to several tens of Gy with an accuracy of 30% [17].

Human fingernails similarly to bird feathers are composed mainly of α-keratin protein which has an alpha-helical tertiary structure. Keratin can be converted to its non-cross-linked form by oxidation or reduction. During this process, cystine residues are transformed to either cysteic acid or cysteine. The cysteine-containing

proteins are call "kerateines". Owing to the presence of cysteine residues, keratin contains a large number of disulphide bonds cross-linking the protein chains. The efficient photodegradation of keratin upon exposure to sunlight is due to the presence of significant amount of the aromatic amino acids – tryptophan and tyrosine. In keratin there are also minor quantities of lipids which contain melanin granules. The nail matrix contains melanocytes in the two lowest cell layers which donate pigment to keratinocytes. Melanin is mainly produced as a result of oxidative polymerization of phenolic compounds by tyrosine or catecholamines which are transformed into pigmented polymers by tyrosinase or peroxidase [6, 11, 14].

As the result of keratin degradation by UV or γ-irradiation free radicals are formed which can be recorded by electron paramagnetic resonance spectroscopy.

In this paper we report the results of comparative EPR studies on free radicals generated in γ-irradiated samples of powdered feather keratin and human fingernails in natural state in the temperature range 77–293 K. The possible application of human fingernails for retrospective EPR dosimetry is also discussed.

Experimental

Feather keratin powdered samples were obtained from the Institute of Biopolimers and Chemical Fibres, Łódź, Poland. Fingernails were collected from healthy adult male and female volunteers and obtained from the Central Laboratory for Radiological Protection (CLOR, Warsaw).

The samples were irradiated at two different temperatures, in liquid nitrogen at 77 K and at room temperature. For the low temperature irradiation, the powder samples of feather keratin and fingernails with a weight of 30 mg were evacuated and irradiated with the dose of 1 kGy in a ^{60}Co gamma source "Issledovatiel" produced in Russia. The room temperature samples were irradiated in the presence of air with lower doses in the range 2–35 Gy using a ^{60}Co source "Mineyola" produced in the Institute of Nuclear Chemistry and Technology, Warsaw, Poland. The radicals generated by radiation were studied over the temperature range 77–293 K by an electron paramagnetic resonance technique using a Bruker ESP-300 spectrometer operating in the X-band (9.5 GHz), equipped with a cryostat with a variable temperature unit, frequency counter (Hewlett Packard) and a Bruker gaussmeter. For precise *g*-value determination, calibration with 2,2-di(4-*t*-octylphenyl)-1-picrylhydrazyl (DPPH) at 0.1 mW (g = 2.0036) was carried out.

Results

Room temperature EPR study of feather keratin and human fingernails

The room temperature EPR measurements of non-irradiated samples of feather keratin and fingernails show a weak isotropic singlet with g_0 = 2.005 and the width ΔH_{pp} = 0.95 mT (Figs. 1a and 2a). After γ-irradiation at room temperature, a similar singlet but of higher intensity is

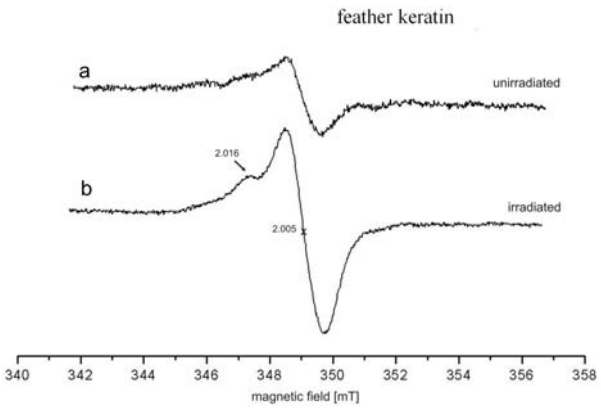


Fig. 1. Experimental EPR spectra of powder feather keratin irradiated and recorded at room temperature (a) non-irradiated samples, (b) γ-irradiated samples (D = 10 Gy). The attenuation of EPR signal is the same.

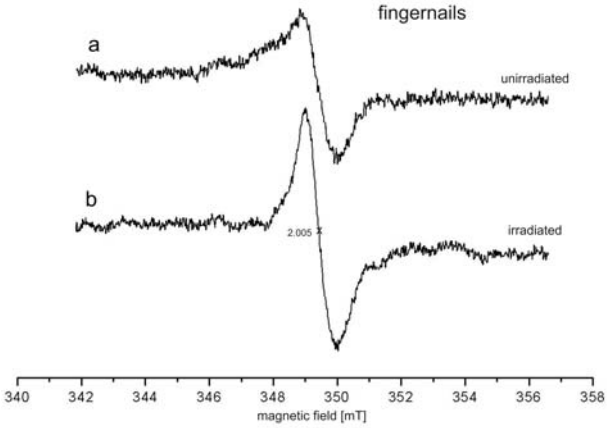


Fig. 2. Experimental EPR spectra of human fingernails irradiated and recorded at room temperature (a) non-irradiated samples, (b) γ-irradiated samples (D = 10 Gy). The attenuation of EPR signal is the same.

recorded (Figs. 1b and 2b). Additionally, the low-field part of a singlet observed in feather keratin is overlapped with a much weaker unidentified signal with g = 2.016. This signal is not seen in the spectra of fingernail samples irradiated at room temperature. The radiation-induced signals in both samples are not stable at room temperature and decay markedly during the first days after irradiation. The intensity of EPR signal of the fingernail sample irradiated with a dose of 35 Gy is lowered by a factor of 2 after 5 days of storage but after the next 25 days the intensity of the signal remains still slightly higher than that of a non-irradiated sample (Fig. 3).

The dose dependence of the EPR signal (g_0 = 2.005) intensity has been studied for fingernail samples in the range of 5–35 Gy. Five fingernail samples were irradiated with five increasing doses of 5, 10, 15, 25 and 35 Gys while the EPR measurements were carried out 5 days later. The signal intensity was measured as peak-to-peak height of the singlet with g_0 = 2.005. The relationship between signal intensity and radiation dose (Fig. 4) is nearly linear in the range 5–15 Gy, however, deviation from linearity above 15 Gy is observed. Although we measured the samples irradiated with the doses lower than 5 Gy the results of this measurements are not presented here because the EPR signal intensity 5 days

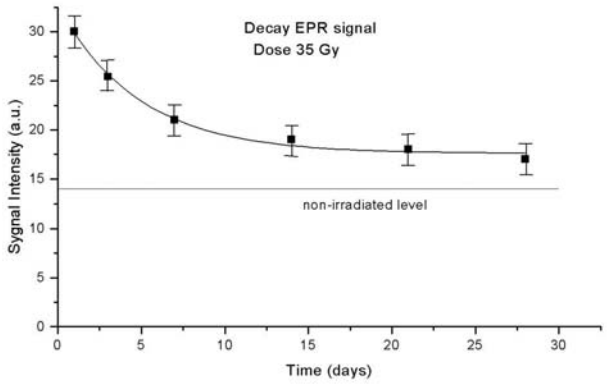


Fig. 3. Decay of EPR signal in γ-irradiated at room temperature (D = 35 Gy) human fingernails.

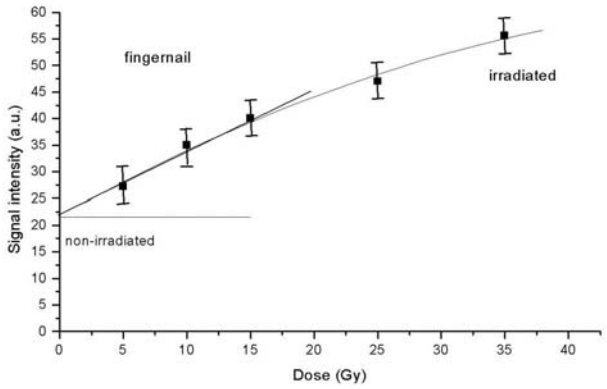


Fig. 4. Dose-response curve for the intensity of EPR signal radiation-induced in γ-irradiated at room temperature of human fingernails.

after irradiation was found very close to the intensity of these signal recorded with non-irradiated samples.

Low temperature EPR study of feather keratin and human fingernails

The EPR signal of the sample of powder feather keratin irradiated and recorded at 77 K is presented in Fig. 5a. It is a broad complex signal in which the main component is an anisotropic singlet with g-values: $g_1 = 2.024, g_2 = 2.011$ and $g_3 = 1.997$ representing a sulphur-centred radical. On warming at 250 K, the spectral component at $g_1 = 2.024$ decreases and a weak anisotropic signal with $g_1 = 2.056$, $g_2 = 2.011$ and $g_3 = 1.997$ appears (Fig. 5b). After warming to room temperature, another weak spectral component at $g = 2.030$ is recorded which seems to be associated with another sulphur-centred radical (Fig. 5c). However, the most intensive signal at room temperature is a singlet with $g = 2.005$ overlapped by the signals representing sulphur-centred radicals. They decay quickly at room temperature and after one hour a singlet with $g = 2.005$ dominates the EPR spectrum entirely. On its low-field part the inflexion at $g = 2.016$ is observed representing an undefined stable radical. Exactly, the same spectrum is recorded after irradiation of feather keratin at room temperature (Fig. 1).

The EPR spectrum of human fingernails irradiated at 77 K looks very similar as the spectrum of feather keratin at the same temperature. The g-values of the anisotropic

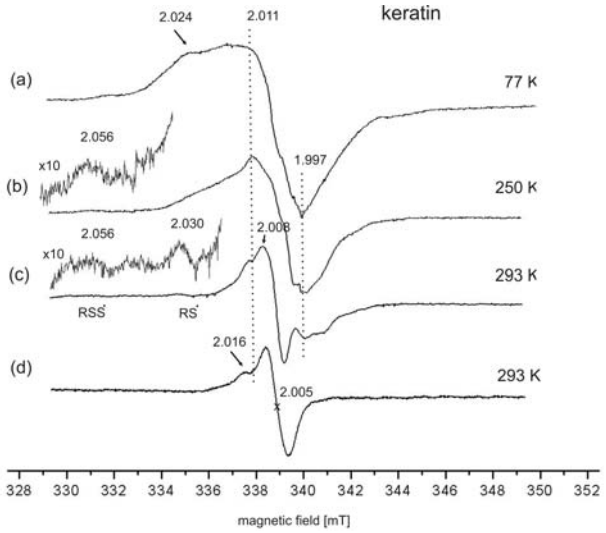


Fig. 5. Experimental EPR spectra of feather keratin powder γ-irradiated at 77 K and measured at (a) 77 K, (b) 250 K, (c) 293 K, (d) 293 K – after 5 days in air.

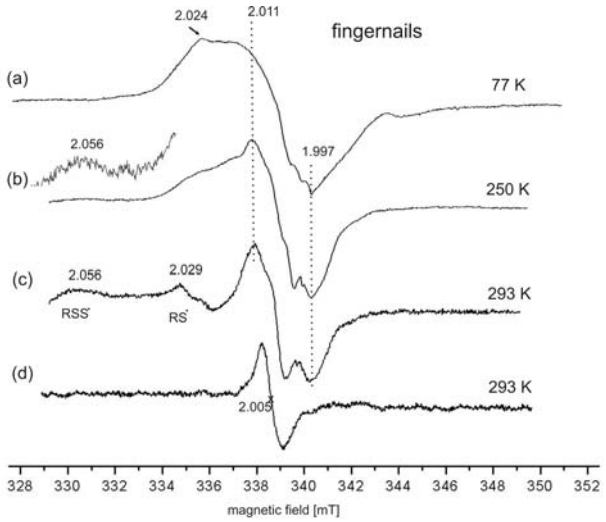


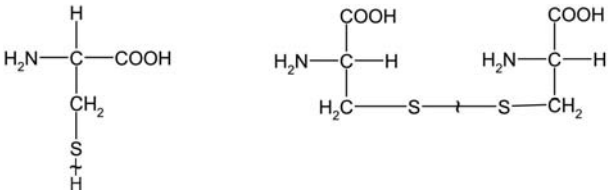
Fig. 6. Experimental EPR spectra of human fingernails, γ-irradiated at 77 K and measured at (a) 77 K, (b) 250 K, (c) 293 K, (d) 293 K – after 5 days in air.

singlet are exactly the same as for the keratin sample. Also the spectral transformations during thermal annealing to room temperature are practically the same in both samples (Fig. 6).

Discussion

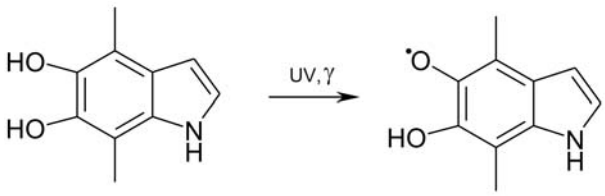
The earlier articles about radiation-induced radicals in human nails and hair focus mainly on the possible application of stable EPR signals for the retrospective dosimetry [2, 4, 15, 17].

The knowledge of the low temperature processes which may transform the primary radicals in these materials to the radicals recorded at room temperature is very fragmentary based entirely on the studies of much simpler molecules [1, 9, 16, 19]. Our low temperature EPR results complete the information about free radicals generated radiolytically in keratin and fingernails and their transformations during thermal annealing.



Cysteine Cystine

Scheme 1.



Melanin Semiquinone radical

Scheme 2.

Human nail samples as well as human hairs contain two major components: protein-keratin and biopolymer-melanin. The same components are found in feather keratin. The free radicals can be induced by radiation in both components and we identified two types of radicals – the first ones which are unstable at room temperature and the second ones which can be recorded at room temperature over a month period.

The EPR parameters of signals unstable at room temperature suggest that they represent the sulphur-centred, thiyl type radicals. They can be formed as a result of radiolysis of cysteine or cysteine residues which are abundant in keratin protein. It is proposed that the cleavage of the disulphide bond in cysteine or S-H in cystine (Scheme 1) leads to the formation of sulphur-centred radicals in both aminoacid residues [6, 10].

The radiation generated sulphur-centred radicals have been studied in numerous sulphur containing compounds [1, 16] however they are identified for the first time in γ-irradiated fingernails and feather keratin in our present work.

The EPR singlet with g values $g_1 = 2.024$, $g_2 = 2.011$ and $g_3 = 1.997$ recorded at 77 K was interpreted in a different way by different research groups [1, 10, 16]. We have assigned it to the $RS^{\bullet}$ thiyl radical with the unpaired electron located on the cysteine residue in protein-keratin. Millington [10] recorded a similar spectrum in cysteine γ-irradiated at 77 K and concluded that it represents $RSSR^{+\bullet}$ radical cations in cysteine. In the same way Symons [16] interpreted a similar EPR spectrum with similar g-values observed at 77 K in γ-irradiated disulphides and thiols and assigned it to the combination of EPR signals of cystynyl radical cations and anions, $RSSR^{+\bullet}$ and $RSSR^{-\bullet}$ [16]. After warming irradiated keratin or fingernail samples to 250 K, a new singlet with g-values: $g_1 = 2.056$, $g_2 = 2.011$ and $g_3 = 1.997$ is recorded (Figs. 5b and 6b). It can be assigned to perthinyl radicals which are formed by the reaction of $RS^{\bullet}$ thiyl radical with a cysteine molecule in the absence of oxygen [1, 16, 19].

$$RS^{\bullet} + RSH \rightarrow RSS^{\bullet} + RH$$

The sulphur-centred radicals in keratin and fingernail samples decay completely after a few hours at room temperature and then a singlet with $g = 2.005$ and $\Delta H_{pp} = 0.95$ mT is observed (Figs. 5d and 6d). The same signal is recorded in non-irradiated samples of keratin and fingernails and after irradiation at room temperature, but then its intensity is distinctly lower. The EPR parameters and its stability suggest that it originates from melanin which is the component of feather keratin as well as fingernails. Melanin is the only known biopolymer containing a population of intrinsic, semiquinone-like radicals [8, 12]. Additionally, extrinsic free radicals are photo-generated in melanin by UV and visible light. This is why non-irradiated samples of keratin and fingernails show the EPR signal. The ionizing radiation produces the same semiquinone free radicals in the following reaction (see Scheme 2).

Melanin ensures the only natural photoprotection in skin by decreasing the population of free radicals generated by UV. As a photoprotector, it acts in both ways as a sunscreen and radical scavenger [3]. In feather keratin and human fingernails the radicals generated by irradiation can be also scavenged by melanin, however, as one can see in Fig. 6 some population of radicals generated by radiation survives and can be used for the retrospective estimation of radiation dose with the use of EPR spectroscopy.

Till now the best material for EPR dosimetry is biological hydroxyapatite from tooth enamel and bones. The radiation-induced EPR signal in these materials is very stable and characteristic of irradiated samples. The intrinsic signal in non-irradiated samples is very small and different in shape than the extrinsic one. Owing to that, the detection level is relatively low – 0.5 Gy for tooth enamel and 2 Gy for compact bones [5]. Use of EPR dosimetry with tooth enamel was recommended by the IAEA for retrospective dose assessment [7] especially to measure the accumulated dose resulting from intake of bone-seeking radionuclides, e.g. ^{90}Sr. There are strong indications based on international inter-comparison that tooth enamel EPR dosimetry gives correct and accurate dose assessment. However, there are some shortcomings due to the necessity of teeth extraction and the difficulties with the separation of tooth enamel from dentine.

Those drawbacks do not exist with EPR dosimetry of fingernails, but other factors cause strong limitations for the applications of this methodology to dose assessment. First, the intensity of intrinsic signals for samples from various individuals differs even in 50%. Additionally, the radiation-induced signal is not specific for irradiated fingernails – the same signal with lower intensity is recorded in non-irradiated samples. Finally, it decays rather quickly during the 5 days after irradiation to remain only slightly higher than the intensity of non-irradiated samples after 30 days. Because of that the detection level for the fingernail EPR dosimetry is much lower than that for the tooth enamel dosimetry. According to our rough estimation, this level is about 5 Gy, but even for higher doses the assessment will not be very accurate. Therefore, the fingernail EPR dosimetry can be applied only to some specific radiation accidents as, for example, the assessment of dose adsorbed in hands of the workers in nuclear laboratories. In such cases the fingernail EPR dosimetry can be the method of choice.

Conclusions

The low temperature γ-irradiation of feather keratin and human fingernails leads to the formation of two types of radicals – the sulphur-centred thiyl radical with the unpaired electron on cysteine residue and the semiquinone type radical formed by hydrogen atom abstraction from a hydroxyl group of melanin molecule. Upon thermal annealing the thiyl radicals are transformed to perthinyl radicals which are not stable at room temperature. In contrast, the semiquinone melanin radicals can be recorded at room temperature. They slowly decay reaching the level of an intrinsic EPR signal after 30 days. It is concluded that the EPR signal of semiquinone melanin radicals generated by ionizing radiation in fingernails can be used for rough assessment of doses above 5 Gy. The fingernail EPR dosimetry seems to be especially useful for assessment of doses absorbed in hands of nuclear laboratories personnel working with radioisotopes of high activity.

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