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High-field/ high-frequency EPR study on stable free radicals formed in sucrose by gamma-irradiation

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Abstract
The EPR spectrum of sucrose irradiated by high-energy radiation is complex due to the presence of more than one radical species. In order to decompose the spectrum and elucidate the radical magnetic parameters a high-field (HF(-)EPR) study on stable free radicals in γ-irradiated polycrystalline sucrose (table sugar) was performed at three different high frequencies—94, 190 and 285 GHz as well as at the conventional X-band. We suggest a presence of three stable radicals R1, R2 and R3 as the main radical species. Due to the increase of g-factor resolution at high fields the g-tensors of these radicals could be extracted by accurate simulations. The moderate g-anisotropy suggests that all three radicals are carbon-centred. Results from an earlier ENDOR study on X-irradiated sucrose single crystals (Vanhaelewyn et al., Appl Radiat Isot, 52, 1221 (2000)) were used for analyzing of the spectra in more details. It was confirmed that the strongest hyperfine interaction has a relatively small anisotropy, which indicates either the absence of α-protons or a strongly distorted geometry of the radicals.

Keywords: *High field/high frequency EPR, γ-irradiated sugar, organic free radicals*

Introduction

Several aspects contribute to the interest in studying of free radicals formed in sucrose under high-energy irradiation:

- These radicals are stable in the time after irradiation, and therefore sucrose can be used as a material for dosimetry. In addition this compound has the advantage to be universally available, cheap and easy to handle, and tissue equivalent. Furthermore it shows a linear EPR response in a wide range of doses [1–3].
- Sucrose and its constituent monosaccharides—glucose and fructose are very important from a biological point of view. Recent EPR studies on the radiation effects on biological objects showed a formation of sugar radicals as a result of this treatment [4,5].
- On the other hand the EPR technique has been used as an analytical method for detection and control of radiation treatment of food. Concerning the EPR investigation on irradiated dried fruits, obtained spectra are due to radicals of different sugars, formed in the radiation process [6].

The EPR spectrum of high-energy irradiated sucrose is complex due to the formation of more than one free radical species in the irradiation process. EPR studies have been performed so far mainly at the

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standard X-band frequency and these measurements do not provide satisfactory information about the number and magnetic parameters of the radicals [7–10]. In the case of such kind of complex spectra the use of high field/high frequency-EPR (HF-EPR) at a few different frequencies is a powerful tool for the identification and separation of the different species. The main advantage of HF-EPR is the increase of *g*-factor resolution that leads to simplification of the registered spectra. The use of several EPR frequencies is necessary because of the large number of magnetic parameters, in particular hyperfine couplings, in organic radicals. This approach has already been used to identify the radicals produced in irradiated β-blockers [11] and for studying systems relevant for dosimetry and dating [12]. In both cases the objects of investigation are composite spectra due to more than one paramagnetic species in the sample.

The main purpose of this first HF-EPR study on γ-irradiated sucrose is to separate the different kind of free radicals, to determine their relative concentration, and to attempt assignment of their structures. This requires elucidation of their magnetic parameters (*g*-tensors and hyperfine tensors). The broader aim is a better understanding of the radiation chemistry of this material.

The paper is organized as follows. First, we consider whether the spectra of the different species can be separated via the electron spin relaxation time either by relying on temperature dependence of the total spectrum or by saturation effects in continuous-wave (CW) EPR. Second, we consider the multi-frequency approach for separation and discuss its advantages and limitation for the case at hand. Third, we attempt to simulate the CW-EPR spectra at three high frequencies. Based on these simulations we finally quantify the relative contribution of the radicals and discuss possible structures.

Materials and methods

Material

This study concentrates on common white table sugar, as this is the material of interest for dosimetry in the aftermath of a radiation accident. Reproducibility of γ-radiation induced EPR spectra in table sugar samples from different countries has been demonstrated before [13] and it was also shown that radiation characteristics of table sugar and pure sucrose are essentially the same [14]. Throughout this paper we, therefore, refer to the material, which consists of 97–99% sucrose, by the chemical term sucrose. The white table sugar was purchased from the local supermarket. It was ground in an agate mortar to a very fine powder before irradiation. The powder had to be very fine in order to avoid the presence of larger crystalline pieces in the samples leading to incomplete powder averaging and thus to an angle-dependent EPR spectrum especially at high frequencies. It was checked that the spectrum did not change with time due to uptake of water from the air.

Irradiation

Sucrose powder, placed in a polyethylene bag, was γ-irradiated at room temperature with the dose of 7.5 kGy using a ^{60}Co γ-irradiation unit INRNE (Institute for Radio Nuclear Energetic). The dose rate was 0.36 kGy/h.

Methods, instrumentation and procedures

Multi-frequency CW-EPR. EPR spectra of γ-irradiated sucrose were recorded at four different frequencies— 9.73, 94, 190 and 285 GHz.

The spectra at 190 and 285 GHz were registered on the HF-EPR spectrometer operating at the High Frequency-High Field Techniques laboratory in Pisa (Italy), equipped with solid state sources (RPG, Germany) delivering monochromatic radiation at three frequencies: 95, 190 and 285 GHz, a superconductor magnet (Oxford Instruments, UK) with a maximum field of 12 T, a home made ultra-wide band probe head, and a hot electrons bolometer detector (QMC, UK). A CF1200 (Oxford Instr., UK) continuous flow cryostat was used for temperature control from 310 K down to liquid helium temperature. Approximate microwave power control was performed in these experiments by measuring the output voltage of the bolometer. Spectra at 285 GHz frequency were recorded at room temperature with the following optimal parameters: bolometer voltage— 15 V, modulation amplitude (MA)—ranging from 1 to 0.1 G, modulation frequency (MF)—10 kHz, sensitivity (SE)—3 mV, time constant (TC)—300 ms. The spectra at 190 GHz were registered in the temperature region 300–5 K with optimal parameters: MA—1 G, MF—10 kHz, TC—1 s. SE was increased from 1 to 3 mV with the decrease of the temperature. Microwave power (MP) was decreased for low temperature measurements because of saturation effects by reducing the bolometer signal from 18 V at 300 K to 20 mV at 5 K.

Some of the CW-EPR experiments at 94 GHz were performed at the Laboratory of Molecular Magnetism, University of Florence, Italy on a spectrometer Bruker Elexsys E600 equipped with a 6 T split-coil superconducting magnet by Oxford Instruments. A Bruker Teraflex resonator with a cylindrical cavity operating in the TE011 mode was employed. For variable temperature measurements between 4 and 300 K a continuous flow ^{4}He cryostat by Oxford Instruments was used. The remaining experiments at the same frequency were performed on a Bruker E 680 spectrometer with the same type of probe head,

the same type of cryostat, and a 5 T split-coil superconducting magnet by Magnex. Optimal experimental parameters were: Temperature—100 K, MF—100 kHz, MP—lower than 5.012×10^{-8} W, MA—1 G, TC—5.12 ms, Sampling time—20.48 ms. Series of EPR spectra were registered at 94 GHz and room temperature using microwave power in the interval from 7.92×10^{-7} to 9.98×10^{-5} W.

X-band EPR spectrum was registered on X-band Bruker ER 200D at room temperature using a standard rectangular cavity (ER 4102ST) working in TE102 mode.

For the measurements on both W-band (94 GHz) spectrometers, an external calibration of the field axes was performed according to the procedure described in the manual of the spectrometers, using a Mn(II):CaO standard sample provided by Bruker. The field calibration and the exact frequency at X-band were controlled, using a Mn(II):MgO standard ($g = 2.031$ for the third line in Mn(II) spectrum). For the higher frequencies an indirect calibration with respect to the 94 GHz values was performed, using as a marker a feature from the sucrose spectrum that can be clearly identified at 94, 190 and 285 GHz (designated by arrows in Figure 2).

All EPR measurements were carried out at least one week after the irradiation because a previous study at X-band [15] showed that the EPR signal of γ-irradiated sucrose undergoes changes in the first few days after irradiation. After reaching the steady state, the spectrum is stable at least for about 1 year.

Simulations and theoretical calculations. The principal axis system of each *g*-tensor was defined as the molecular coordinate system of the respective radical. The orientation of the principal axis system of each hyperfine tensor is the characterized by a set of three Euler angles, which have to be determined by systematic variation. For a given set of Euler angles, the components of the hyperfine tensor in the molecular frame were computed by a subroutine of the EasySpin package [16]. These values were then used as input data for simulations of the powder EPR spectra by the Bruker WINEPR (SimFonia) program [17]. Identical parameter sets were used for simulations of the spectrum of a given radical spectra at all three high frequencies—94, 190 and 285 GHz as well as at the conventional frequency of 9.73 GHz. The final simulated spectrum was then obtained by adding the spectra of all component radicals, varying their relative concentration so that the best fit was obtained. Fits were optimized by visual inspection, as global minimization of the r.m.s. error is not feasible for the case at hand. For the spectrum at 94 GHz, the fit quality was judged by a numerical method as described in the text. The determined sets

of Euler angles are not necessarily unique solutions of the problem.

Density functional theory (DFT) computations were performed with the program ADF 2004.1 [18], using the BLYP functional, which unlike B3LYP allows for the use of Slater-type basis sets. Spin-restricted computations with the TZP basis set (triple-zeta quality with one set of polarization functions) and frozen first shells for carbon and oxygen were used for geometry optimization. Hyperfine couplings and *g*-tensors were computed by spin-unrestricted computations with TZ2P all-electron basis sets (triple-zeta quality with two sets of polarization functions), using the auxiliary program CLGEPR [19] for the *g*-tensors.

Results and discussion

Spin-lattice relaxation effects

The most successful separation of the contributions to the EPR spectrum of high energy irradiated sucrose to date was based on electron nuclear double resonance (ENDOR) of single crystals [20]. Although, such an approach would in principle allow for the quantification of the relative contributions of the radicals, this would be very tedious and was not attempted in this study. In general, such a separation of radicals by single-crystal ENDOR is too laborious for systematically studying the dependence of these relative contributions on radiation contributions or the type of material. Therefore, we try here to find a more simple approach for such a separation.

As the hyperfine tensors are known to be too similar for separation in the EPR spectrum at conventional fields/frequencies, we are left with the relaxation times and the *g*-tensors as possible characteristics for distinguishing the radicals. A combination of better *g*-resolution and relaxation contrast would potentially allow for a relatively simple separation at a single high frequency, possibly even on a commercial HF-EPR spectrometer. We first test whether such an approach is tenable.

We studied the effects on irradiated sucrose EPR spectrum of both—the measurement temperature decrease and microwave power increase. The microwave power dependence (in the range from 7.92×10^{-7} to 9.98×10^{-5} W) of EPR signal of irradiated sucrose recorded at 94 GHz and room temperature showed that the EPR spectrum saturates strongly with the increase of applied microwave power but the broadening is approximately the same across the whole spectrum. The comparison of irradiated sucrose EPR spectra, registered at different temperature (in the range from room temperature to 5 K) and 190 GHz showed that line shapes do not strongly depend on temperature.

Temperature and microwave power effects show that the radicals in the γ-irradiated sucrose sample

have very close spin-lattice relaxation times, which do not allow for radical separation by relaxation. These results were also confirmed by relaxation measurements with pulsed EPR [21].

More details, concerning these experiments are presented in Appendix A.

Multi-frequency CW-EPR study of the irradiated sucrose radicals and simulations

A straightforward way to separate species with different but overlapping *g*-tensors in EPR powder spectra is a systematic study of the dependence of the spectral line shape on the magnetic field. Ideally, at least at the highest frequency used in such a study, the spectral line shape should be dominated by *g*-anisotropy, as this strongly simplifies spectral assignment.

Figure 1 presents the EPR spectrum of *g*-irradiated powder sample of sucrose, recorded at X-band frequency (9.73 GHz) and room temperature. This spectrum is largely unresolved due to broadened overlapping hyperfine lines of the different species and it is not very informative about the radicals giving rise to its features. In previous publications [7–10,20,22] the interpretation of the radical's nature is performed on the basis of similar X-band spectra of both single crystals and powder samples, where at least the *g*-anisotropy is not clear. Only one EPR investigation on X-irradiated sucrose single crystals at Q-band frequencies (35 GHz) [23] allowed for a reasonable estimation of *g*-anisotropy of one of the radicals, but the spectrum is still not well resolved. In these studies there is no agreement about the number and the kind of radicals in the irradiated sucrose sample.

Irradiation of sucrose by different sources gives rise to very similar EPR spectra at X-band [24,25].

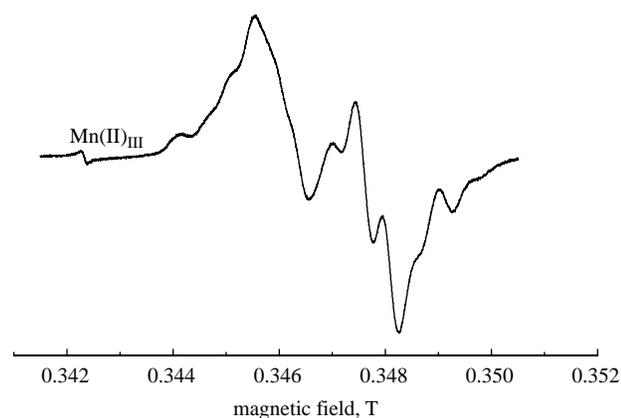For a detailed study of the paramagnetic centres presented in the *γ*-irradiated sucrose sample we performed CW-EPR study of this material at three different high frequencies—94, 190 and 285 GHz. The spectra recorded at these frequencies are plotted in Figure 2. The increase in *g*-factor resolution with frequency clearly reveals the *g*-anisotropy of the radicals present in the sample. This contradicts an earlier hypothesis of an isotropic *g*-factor of radicals in irradiated sucrose [8], and is in an agreement with the assumption of *g*-anisotropy by Gräslund and Löfroth [22] as well as Q-band EPR results of Sagstuen and co-workers [23]. It can be seen from Figure 2 that the EPR spectrum of irradiated sucrose recorded at 285 GHz is dominated by *g*-anisotropy, but yet remains quite complex.

With respect to our aim to distinguish the radicals, two counteracting factors influence the choice of frequency. In the spectrum registered at 94 GHz because of small *g*-anisotropy and close *g*-factors the spectral features still overlap. At this frequency, spectral dispersion due to *g*-factor differences and hyperfine couplings is still of the same order of magnitude. On increasing frequency and thus *g*-factor resolution, however, the hyperfine couplings of the different protons become less resolved due to *g*-strain. Only the largest coupling remains observable.
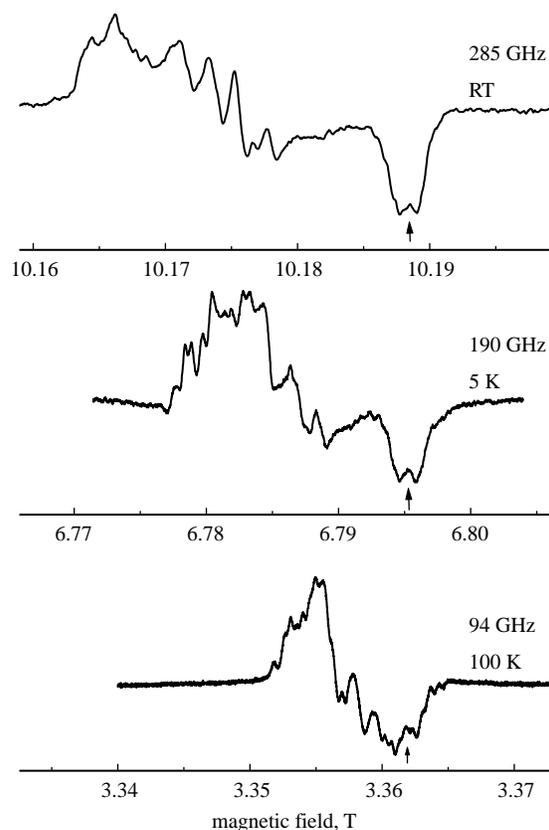

Figure 2.   EPR spectra of *γ*-irradiated sucrose registered at three high frequencies: 94, 190 and 285 GHz. Experimental temperatures are designated on the figure. To show the increase of *g*-factor resolution the spectra are plotted in the same magnetic field sweep range—40 mT. The marker lines in the spectra are designated with arrows.

Figure 1.   X-band EPR spectrum of *γ*-irradiated sucrose, registered at room temperature.

Hyperfine resolution improves slightly at low measurement temperatures, but strong saturation effects at high frequency and low temperature discourage such measurements as a routine tool. More significantly, the improvement in hyperfine resolution at low temperature is not sufficient for the decomposition of the spectra. Consequently the hyperfine couplings cannot be elucidated from CW-EPR spectra and a use of an additional method is necessary.

Sagstuen and co-workers (1986) [23] carried out a room temperature EPR and ENDOR investigation on sucrose single crystals after X-irradiation at room temperature. They suggest the existence of two radicals in the sample, one of which is dominating, and were able to determine the g-tensor of this dominating radical. Their ENDOR spectra are explained in terms of three strong and two weaker hyperfine interactions in the range higher than 17 MHz. The authors assign these interactions to protons in proposed radical structures, but there remains an ambiguity related to the presence of an ENDOR line with very small intensity, which was tentatively assigned to an α-proton interaction in the second radical. A recent ENDOR and EI-EPR [20] study on X-irradiated sucrose single crystals at room temperature shows that three dominating kind of stable free radicals are formed in similar yields. This study derives the hyperfine tensors of these radicals (S1, S2 and S3) which are moderately anisotropic and suggests a weak g-tensor anisotropy around $g = 2.005$ based on X-band ENDOR and CW-EPR experiments. Although the hyperfine couplings determined by Sagstuen et al. [23] and those obtained by Vanhaelewyn et al. [20] are not entirely dissimilar, they do not match either. The only apparent difference between the two investigations is the measurement temperature. Vanhaelewyn and co-workers recorded their spectra at 60 K, which might improve the balance of relaxation times and thus the accuracy of the obtained CW ENDOR data. Some of the differences may also arise from a temperature dependence of the hyperfine couplings, which is expected due to slight conformational changes with temperature.

Relying on more precise and full information and based on the similarity in the X-band EPR spectra of sucrose irradiated by either X- or γ-rays we assumed that the principal hyperfine constants, derived in the aforementioned study [20], can be used to analyze our EPR spectra obtained at the three high frequencies.

Figure 2 shows that resolution of the g-anisotropy indeed increases significantly with the frequency and as a result different hyperfine couplings can be associated to different g-factors. Using common procedures for analysis of powder EPR spectra [26], we were able to elucidate three g-tensors on the basis of the 285 GHz spectrum. For every one of these tensors we assumed $g_{xx} > g_{yy} > g_{zz}$. In the next step we correlated these g-tensors to the hyperfine tensors determined in earlier work [20]. As collinearity between the g-tensor and hyperfine tensors cannot be expected, we also varied the sets of Euler angles that relate the frame of each hyperfine tensor to the molecular frame defined by the g-tensor. Because of the complexity of the EPR spectrum and the overlapping transitions at 94 GHz it is impossible to obtain the sets of Euler angles with good precision at this frequency. An increase of the frequency leads to spectrum simplification and to a stronger dependence of the spectra on the Euler angles, but the hyperfine splittings cannot anymore be "read" directly from spectral features. By varying the Euler angles we did, however, obtain a single set of magnetic parameters and anisotropic line widths that reproduces the EPR spectra at all frequencies satisfyingly. Principal values of the g-tensors are given in Table I, while Table II gives the estimated hyperfine constants and Euler angles. Note that other combinations of Euler angles that give the same projections of the hyperfine coupling to the principal axes directions of the g-tensor may give fits of similar quality. This kind of ambiguity cannot be resolved on the basis of powder spectra.

Figure 3(a)–(d) displays simulated spectra of the three radicals and the spectrum, obtained as a sum of the R1, R2 and R3 simulations as well as the corresponding experimental EPR spectra, respectively, at X-, W-bands, 190 and 285 GHz.

It is evident from Figure 3(c) and (d) that calculated spectra nicely simulate the experimental ones, since all essential features are reproduced. Simulated R1, R2 and R3 EPR spectra at X- and W-bands do not completely coincide with the experimental ones (Figure 3(a) and (b)). Small peaks in the low and the high field part of the spectra, designated by dotted circles on Figure 3(a) and (b), cannot be reproduced using the parameters from Tables I and II. They could be due to a very small amount of a fourth radical, whose spectrum is hidden behind R1, R2 and R3 spectra at 190 and 285 GHz, and is characterized by a larger hyperfine coupling. Since at X- and W-bands the g-anisotropy is not dominating these signals become visible. On the other hand, the absence of these lines in higher frequency spectra could be a result of lower sensitivity because of considerable reduction of microwave power. In their paper Vanhaelewyn et al. [20] suppose the existence of more than three radicals because of weak lines in the

Table I. Principal values of g-tensors for radicals R1, R2 and R3.

	R1	R2	R3
g_{xx}	2.00658	2.0068	2.00603
g_{yy}	2.00466	2.0040	2.00524
g_{zz}	2.00197	2.0018	2.002

Table II.   Principal values of the hyperfine tensors for radicals R1, R2 and R3 from Ref. [20], given in MHz. Last row contains the Euler angles (in degrees).

	R1			R2			R3		
	H1	H2	H3	H1	H2	H3	H1	H2	H3
A-tensor in MHz. Principal values from Ref. [20]	43.9	13.5	17.8	53.9	13.4	8.3	57.6	13.8	10.1
	42.2	15.8	13.9	21.0	19.5	12.0	19.5	20.1	14.8
	53.1	18.8	2.4	34.3	16.5	16.9	39.5	15.6	16.6
Euler angles in degrees	186	−90	184	170	−90	−90	184	24	30
	88	0	92	−32	0	0	100	95	108
	130	0	115	85	0	0	3	3	−4

ENDOR spectra, which cannot be assigned to radicals S1, S2 and S3. Our experimental data do not allow the identification of the parameter of this additional radical but suggest its possible existence.

Despite the high similarity between the experimental and simulated sucrose EPR spectra apparent by visual inspection, we found it necessary to find a quantitative criterion for estimating how well the spectra are reproduced by the simulations. We calculated the goodness-of-fit (R_{factor}) for the simulated sucrose EPR spectrum at 94 GHz, using the method described by Bernhard [27]. The obtained R_{factor} is 0.052, which is a good value for such complex spectrum.

We assume that every single hyperfine coupling corresponds to a single proton because we used crystalline samples in which protons are fixed and they are not geometrically equivalent. An assumption of equivalent protons is not reasonable in this case because they can only belong to a rotating group in the radical and a decrease in temperature would reduce the rate of rotation. This process would result in significant differences in the spectra recorded at room temperature and very low temperature (5 K), which are not observed (see Appendix A). Furthermore, in single crystal ENDOR at low temperature hyperfine interactions of different protons should give distinguishable constants, at least for the strongest couplings [20].

We simulated the proposed radicals in the following concentration proportions for the four frequencies: R1/R2/R3 = 1:0.6:1 for 9.73 GHz; R1/R2/R3 = 1:0.5:1 for 94 GHz; R1/R2/R3 = 1:0.5:0.67 for 190 GHz and R1/R2/R3 = 1:0.5:0.8 for 285 GHz given by the ratio of their spectral intensities. The different ratios required to reproduce the spectra may suggest some difference in line broadening due to strain effects or differences in saturation behaviour. Furthermore, in high-field CW-EPR using the single pass technique it is often difficult to control finely the spectral phase. For complex spectra consisting of several components, such as those of irradiated sucrose, small phase changes lead to differences in the spectral features and the ratio between the intensities of different lines. In our case the experimental setup at X- and W-bands allows for a more accurate phase control than those at 190 and 285 GHz, so that the spectra recorded at the former frequencies are the ones with the most precise phase and the radical ratios derived from them by simulation can be considered as the most accurate ones. At any rate, due to the possible phase error and the uncertainty in the anisotropic line widths used in our simulations we refrain from an exact quantification of the radicals R1/R2/R3. We may conclude, however, that radicals R1 and R3 are present in almost equivalent quantities with perhaps a slight excess of radical R1. The concentration of R2 is about two times smaller than those of R1 and R3.

The radical's nature—comparison with unambiguously assigned literature structures and DFT computations

The nature of radicals in irradiated sucrose is not unambiguously assigned in literature. To improve understanding of this question, we compared the data from previous studies with our experimental results and performed DFT computations on possible radical structures. We obtained experimental g-factor ranges of: 2.00658/2.00197 (Δ ($g_1 - g_3$) = 0.00461), 2.0068/2.0018 (Δ ($g_1 - g_3$) = 0.005), and 2.00603/2.0020 (Δ ($g_1 - g_3$) = 0.00403). The very close g-anisotropy of all three radicals strongly suggests a similar structure, while the relatively small g-anisotropy indicates that very little spin density is located at oxygen atoms.

In the following we refer to the structure of sucrose given in Scheme 1. Concentrating first on carbon-centered radicals, every carbon atom in the sucrose molecule is a possible site of a free radical formation after irradiation. Different structures of carbon-centered radicals are suggested in the literature. Some of these structures are plotted in Scheme 2.

Shields and Hamrick [8] proposed radical structure 1, in which the unpaired electron interacts with one α- and two β-protons. As noticed by these authors, an almost isotropic g-tensor with principal values close to the one of DPPH is expected for this structure. Similar structures (4 in Scheme 2) are suggested by Vanhaelewyn and co-workers [20]. In principle for all organic radicals, in which the unpaired electron is rather strictly localized on a carbon atom, small
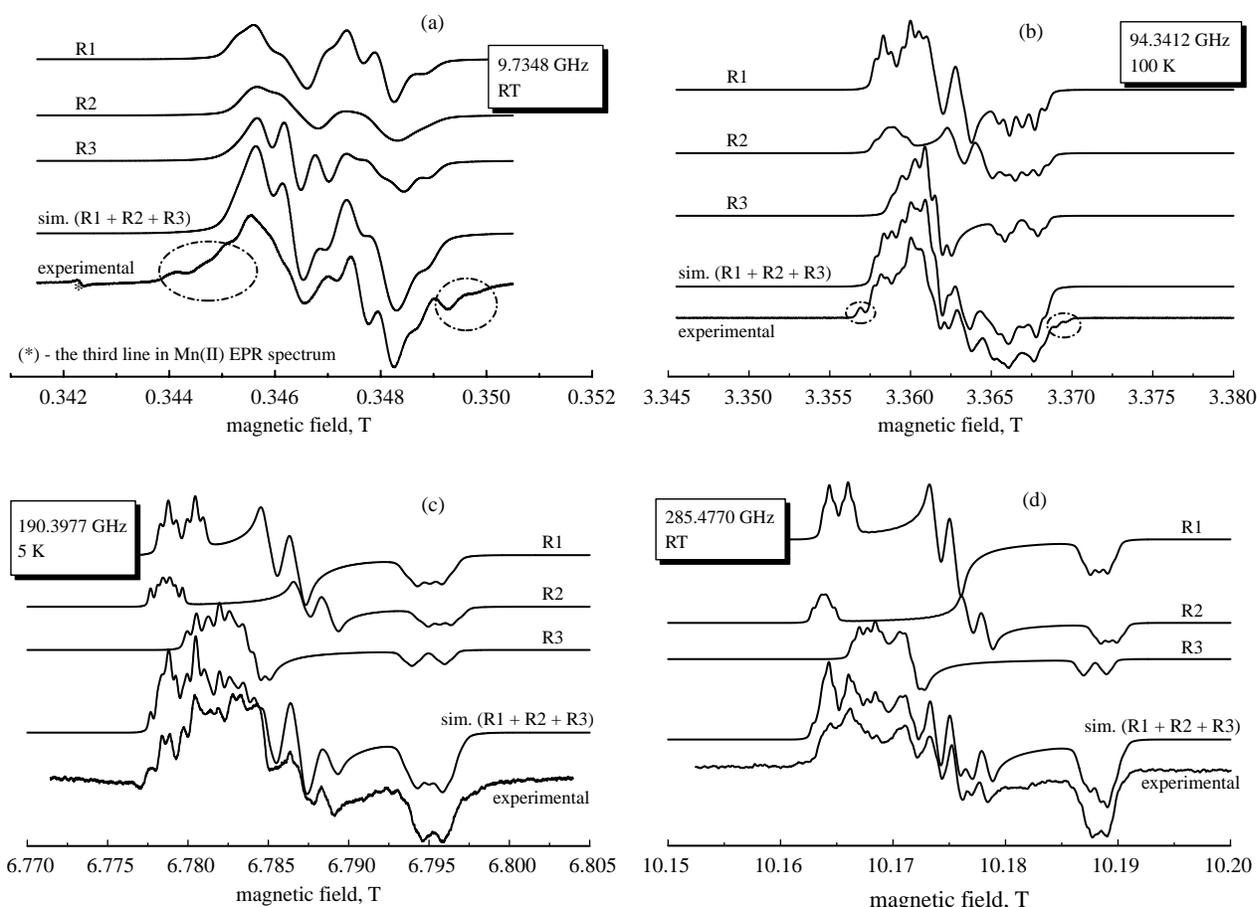
Figure 3. Simulations of R1, R2 and R3 EPR spectra as well as the total (sum) at the four used frequencies, and a comparison with the experimental ones. The exact experimental frequencies were: 9.7348, 94.3412, 190.3977 and 285.477 GHz and simulations in a, b, c and d correspond to them. Anisotropic linewidth tensors (in Gauss, coaxial with the *g*-tensor) for the three radicals and different directions were used: X-band—R1 (3.10, 3.40, 3.50), R2 (4.00, 3.80, 4.80), R3 (5.50, 3.40, 3.00); 94 GHz—R1 (3.40, 4.00, 4.10), R2 (4.00, 3.80, 4.40), R3 (4.70, 3.60, 3.50); 190 GHz, respectively (3.20, 4.50, 6.20), (2.20, 4.00, 5.50), (3.40, 4.20, 4.50), and for 285 GHz (4.10, 4.80, 6.00), (3.60, 4.30, 5.50), (4.60, 5.00, 4.80).For the line shape the ratio Lorentzian/Gaussian was 0.6 or 0.7.

g-anisotropy is expected because of low spin-orbit coupling values. Duboc-Toia et al. [28] investigated by very high field EPR glycyl radical (a typical aliphatic carbon-centered radical) in enzymes and they found *g*-factors in the range: 2.0042/2.0022 and total width of the spectrum: $\Delta(g_z - g_x) = 0.0019-0.0023$ for three different enzymes. Obviously the radicals in irradiated sucrose are not of the same type because of the experimentally observed larger *g*-anisotropy. DFT computations of such radicals (structures 1 and 4) also result in *g*-tensor principal values of approximately (2.002, 2.003, 2.004), i.e. in smaller *g*-anisotropy than is observed experimentally. Furthermore, such radicals derived by hydrogen abstraction from glucose or fructose contain either at least one α-proton with a difference of 60 MHz between the largest and smallest principal value of the hyperfine coupling or at least one β-proton with an isotropic hyperfine coupling significantly larger than the one observed for radical R1. Neither of these features is seen in the experimental spectra.

The difference between computed and experimental values, in particular for the hyperfine couplings, appears to be too large to be attributed to a deficiency of the DFT computations. These discrepancies are confirmed by earlier published result from DFT computations on aliphatic carbon-centered radicals in irradiated glucose [29] and fructose [30], where the calculated hyperfine constants are not in agreement
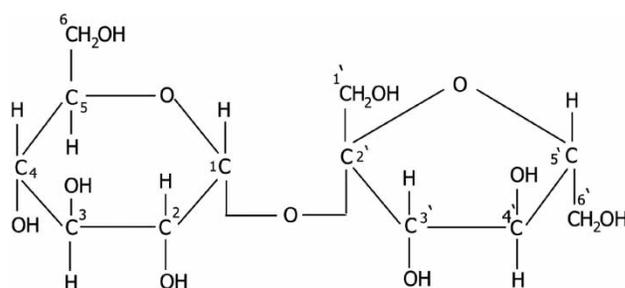
Scheme 1.
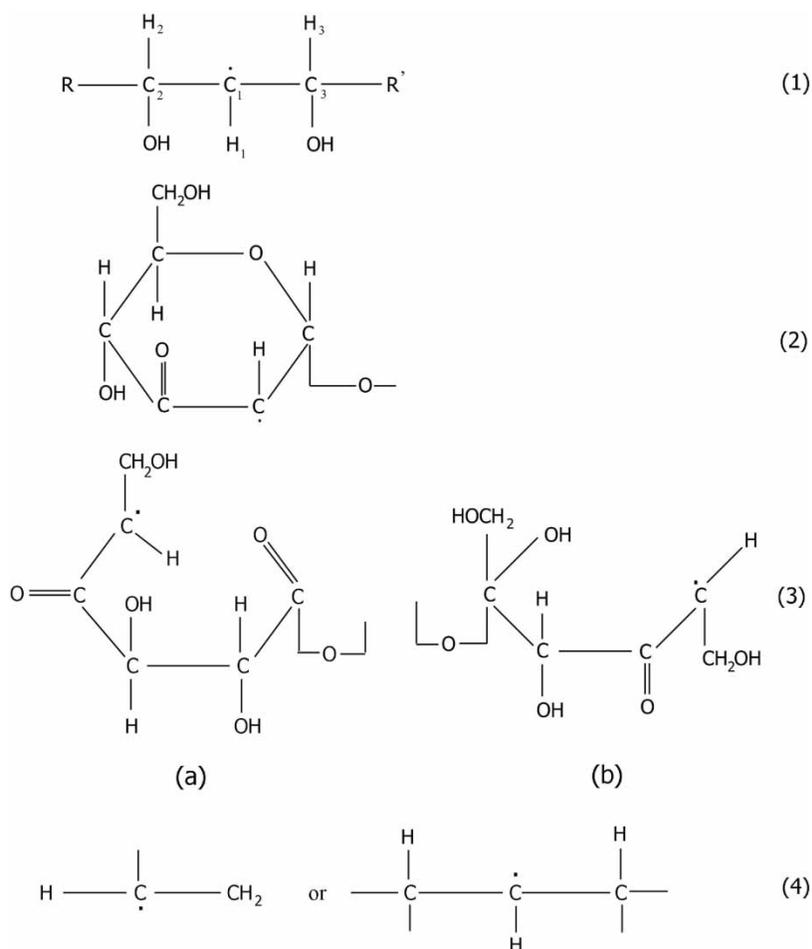
Scheme 2.

with the experimentally defined ones for irradiated sucrose.

Gräslund and Löfhorth [22] studied γ-irradiated sucrose single crystals and they suggested a radical structure with a formation of carbonyl group, corresponding to structure 2 in Scheme 2. In such radical the unpaired electron interacts with an α-proton and a β-proton, giving rise to a quartet EPR spectrum due to one stronger and a second weaker hyperfine splitting. This assumption is not confirmed by the later results [20,23]. From the theoretical study we found that this structure relaxes to a point were part of the spin density distributes to the carbonyl group. According to the DFT computation, the difference between the largest and smallest α-coupling principal component in this case reduces to 50 MHz. The reduction in anisotropy is not as strong as for the open-ring structures, as the strain in the ring prevents a completely planar $-C(=0)-CH-$ arrangement.

As a result of their EPR experiments at X- and Q-band and ENDOR experiments at X-band on X-irradiated sucrose single crystals, Sagstuen and co-authors [23] suggested the existence of open ring radical structures, containing a carbonyl group (corresponding to structures 3 in Scheme 2). Three strong hyperfine couplings are typical for these structures due to the interaction of the unpaired electron with one α- and two β-protons. The g-tensor of one of the radicals determined in this study (2.0067, 2.0049, 2.0027) is close to the values obtained by our experiments. Indeed the presence of carbonyl group close to the carbon-center leads to a delocalization of spin density and, therefore, to a larger spin-orbit coupling causing the observed larger g-anisotropy. DFT computations of such structures yield g-tensors that fit the observed values better than for the other suggested structures. For instance, for the geometry optimized structure 3a in Scheme 2 we compute principal g-values (2.0074, 2.0052, 2.0023) that match our and Sagstuen's experimental values as good as or even better than can be expected considering the limited precision of g-tensors obtained from established DFT approaches. Likewise, the computed principal values of the hyperfine tensor (-57.7, -37.6, -12.1 MHz) for the α-proton are in reasonable agreement with the experimental values of Vanhaelewyn et al. [20] for radicals R2 and R3 used in our spectral simulations (Table II). The remaining

deviations can also stem from imprecision of the DFT computation, but might also derive from incomplete relaxation of the structure of the radical created by ring opening. As the relaxed structures of such radicals differ strongly from the structure of the parent molecule, complete structural relaxation may be impossible in a crystal.

The decreased hyperfine coupling of the α-proton compared to structures 1, and 4 in Scheme 2, is also expected from basic considerations. In structures 3a and 3b the unpaired electron is delocalized over the sp^2 carbon and the carbonyl group orbital. An assignment of radicals R2 and R3 found in this study to structures similar to structure 3 in Scheme 2 thus appears to be feasible. It is worth noting that in an UV spectroscopic investigation of γ-irradiated sucrose by Flores and co-workers two bands were found in the region around 250 and 320 nm, whose intensities increase with the dose of irradiation, and which were associated with carbonyl groups [25]. Although, this and the rather nice agreement of computed and experimental magnetic parameters strongly support our assignment, we cannot conclude that it is the only possible one for the structures of radicals R2 and R3.

Radical R1 presents a problem in that its largest hyperfine coupling has a very small anisotropy and thus apparently corresponds to a β-proton rather than an α-proton. Based on the deuteration experiments of Shields and Hamrick [8] as wells as Sagstuen et al. [23] it appears unlikely that any of the strong hyperfine couplings resolved in the X-band EPR spectrum stems from an exchangeable proton, hence we exclude an α-OH group as the source of this coupling. The three observed couplings might then be two β-protons (H1 and H2) and a γ-proton with an unusually large coupling (H3), possibly due to an unusual spatial proximity to the radical site. At present, we cannot suggest a likely structure that would also need to fulfil the condition that the paramagnetic centre has no α-proton.

We also checked other possible radical structures. In the case of oxygen-centered radicals the expected g-anisotropy is considerably higher than the one in carbon-centred radicals because of stronger spin-orbit couplings. Box and Budzinski [31] investigated by EPR and ENDOR methods radicals in sucrose single crystals X-irradiated at liquid helium temperature. They suggest a presence of alkoxyl radical with electron spin density localized on an oxygen atom (O-atom connected to C_3' – atom, see Scheme 1) and g-values distribution from 2.0270 to 2.0037. These radicals were also found to be unstable at elevated temperature. Other study was performed by Bernhard and co-workers [32] on RCH_2O radical in X-irradiated at low temperature single crystals of nucleosides and nucleotides. The g-tensors and hyperfine values for this kind of radicals strongly disagree with the parameters that we used to simulate our spectra

and those published by Vanhaelewyn and co-authors [20]. In principle alkoxy radicals generally exhibit a Δg 10-fold larger than observed here, β-couplings far larger than observed here and are generally unstable above 77 K and therefore are not actual candidates for the room temperature radicals in sucrose. Our own DFT computations of alkoxyl radicals also suggest a much larger g-anisotropy than is observed experimentally. The computed maximum g-value ranges between 2.023 and 2.092 for alkoxyl radicals, derived from glucose. It should be noted that this value is extremely sensitive to hydrogen bonding or any other interaction with neighbour molecules, as such interactions lift the near-degeneracy of orbitals that is responsible for the strong spin-orbit coupling.

Conclusions

HF-EPR spectra of sucrose γ-irradiated at room temperature were found to be dominated by at least three different stable free radicals with rather similar g-tensors and hyperfine couplings. Variation of microwave power and temperature does not allow for separation of these radicals.

In contrast, a multi-frequency approach using measurements at X-, W-bands, 190 and 275 GHz does allow for a satisfactory elucidation of the g-tensors for the three radicals R1, R2 and R3. A more detailed analysis of the spectra was based on results from a previous ENDOR study [20]. For all three radicals g-tensor and hyperfine tensor principal values for the three most strongly coupled protons could be assigned. The moderate g-anisotropy suggests that all three radicals are carbon-centered. Concerning all previously suggested alternatives for the structure of these radicals we found that the most likely structures correspond to ring opening with formation of a keto-group next to the radical centre as proposed by Sagstuen and co-authors [23], but this assignment is still not completely proved.

The quantitative estimation showed that radicals R1 and R3 occur in almost the same concentration, while the concentration of radical R2 is about two times less.

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Appendix A: Spin-lattice relaxation measurements

A.1. Temperature dependence

The measurements of the irradiated sucrose EPR spectra at different temperatures—in the interval from room temperature to 5 K and frequency of 190 GHz showed that all spectral features seen at 5 K are maintained up to room temperature. There are small differences in the line intensities of the low field part where some features are better resolved at low temperature (data not shown). However, a reliable separation of different radical species in irradiated sucrose is certainly impossible on the basis of the temperature dependence of the EPR spectra.

It is important to notice that the spectra at low temperature needed to be recorded at very low microwave power because of saturation problems, suggesting a very low relaxation rate. For a comparison the microwave power reduction corresponds to 18V and 20 mV of the bolometer signal, respectively, for the spectrum recorded at room temperature and for the spectrum recorded at 5 K.

A. 2. Microwave power dependence

When EPR spectra of powder samples containing mixture of paramagnetic species with anisotropic parameters are registered consecutively with an increase of the microwave power, two effects can be expected—different paramagnetic species may show different saturation effects due to a difference in their spin-lattice relaxation times or saturation may be anisotropic due to different relaxation times for one and the same species in different orientations. Lassmann et al. [33] observed the latter effect for protein thiyl radical in disordered systems. Such an approach might lead to simplification of the EPR spectrum of γ-irradiated sucrose and may form the basis for separating different kind of radicals. We tested this at 94 GHz and room temperature to profit from the better resolution compared to X-band (9.5 GHz) spectra and, on the other hand, to avoid the lower sensitivity associated with saturation at very low microwave power that is observed at low temperatures. Common broadening in the EPR lines for all directions and radicals was observed (data not shown). This effect did not allow the spectra of different radicals (contributing to the total EPR spectrum) to be distinguished.