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Fourth stable radical species in X-irradiated solid-state sucrose[†]

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High-energy radiation produces radicals in crystalline sucrose. As such, sucrose is considered as a relevant model system for studying radiation damage to the sugar units of DNA. Many of these radicals are stable, detectable at room temperature with electron paramagnetic resonance (EPR) and their concentration is proportional to the absorbed dose in a considerable range. This makes sucrose also an interesting system for dosimetry. Dose assessment protocols rely on measurements of the total intensity of the EPR powder spectrum, so it is likely that they could be further improved if the composite nature of the spectrum was understood completely. Recently, it was shown that the three known stable radicals can only account for the central part of the spectrum and that features in the wings remain unidentified. In this work, we show, based on the analysis of the powder EPR patterns recorded at three microwave frequencies, that the contribution of one more species is sufficient to explain the entire spectrum. The determination of the spin Hamiltonian parameters is corroborated by a Q-band (34 GHz) single crystal electron-nuclear double resonance (ENDOR) analysis. The chemical structure of the fourth species is explored by analysis of the determined **g** and four ¹H hyperfine (HF) tensors, and verified using density functional theory (DFT) calculations. The ENDOR spectrum of the largest HF interaction of the fourth species was exploited to isolate the radical's absorption-like EPR spectrum from a multicomponent powder pattern.

1 Introduction

Radiation-induced free radicals are well known to be one of the major causes of damage to biologically relevant systems, in particular DNA^{1,2}. The radiation chemistry of DNA has been extensively studied by electron-magnetic-resonance-based techniques, (for a recent review, see Ref. 3). Reaction pathways and composition of the paramagnetic species are rather well established, but it is reasonable to assume that the knowledge can be further improved and then more easily transferred to in-cell conditions if a deeper understanding of the radicals' chemical structures is attained. Unfortunately, information of this kind is extremely difficult to extract by studying DNA directly, because a large number of spectral components are observed simultaneously and single

crystal samples are extremely rare. For these reasons, model systems that mimic specific structural elements of DNA are used. An approach that combines the high resolution of electron-nuclear double resonance (ENDOR) spectroscopy and the high potential of density functional theory (DFT) calculations has proven to be very effective for studying these systems. A number of successful single-crystal carbohydrate studies is available and has recently been summarized by Vrielinck et al.⁴. From the point of view of radiation research, sucrose is one of the more interesting carbohydrates. Not only is it a valid model system for sugar-containing macromolecules, but it is also relevant to dosimetry^{5–7}: its electron paramagnetic resonance (EPR) spectrum is stable for months, it is detectable at room temperature (RT) and its intensity exhibits a linear relationship with the absorbed dose in the 100 mGy–10 kGy range, making it a potential personal dosimeter and a relevant system for characterization of irradiated food-stuffs.

The stable EPR spectrum of sucrose is known for almost sixty years⁸. At first it was believed that only one radical species contributes to the spectrum⁹, but in the mid-eighties Sagstuen et al.¹⁰ have shown that its composition is certainly more complex. Later, Vanhaelewyn and co-workers¹¹ have proved this rigorously

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using statistical analysis. In recent years, three dominant radical species (T1–T3) were thoroughly characterized and identified^{12–14}. After the complete spin Hamiltonian characterization (HF interactions and **g** tensors), it was shown that the stable spectrum is now very well understood except for some features in its wings¹⁵. In the present work we have determined the **g** and four HF tensors of a fourth, up till now unknown, radical species using single crystal ENDOR and ENDOR-induced EPR (EIE) spectroscopy. Moreover, the dependence of its largest HF interaction's ENDOR spectrum on the static magnetic field is exploited to isolate its absorption-like EPR pattern from a multicomponent powder spectrum. The chemical structure of the fourth species is inferred from the determined spin Hamiltonian parameters, and validated using single-molecule *in vacuo* DFT calculations. We finally show that by including this species in the simulations of the powder EPR spectra in multiple microwave frequency bands, all components in the stable radiation-induced EPR spectrum of sucrose are now understood.

2 Materials and Methods

Sucrose (C₁₂H₂₂O₁₁) is a disaccharide, composed of glucose in α -pyranose and fructose in β -furanose forms, respectively. Its crystal structure is monoclinic with space group *P*2₁, with two molecules in the unit cell, related by a twofold screw-rotation around the *b* axis, i.e. $\langle x, y, z \rangle$ relates to $\langle -x, y + 1/2, -z \rangle$. The lattice parameters are known from neutron diffraction experiments¹⁶: *a* = 1.0868 nm, *b* = 0.8710 nm, *c* = 0.7761 nm and β = 102.97°, where β denotes the (monoclinic) angle between the *a* and *c* axes. The *a*^{*}*bc* orthogonal reference frame was chosen in accordance with our previous work, where *a*^{*} is perpendicular to *b* and *c*. For brevity, normal vectors of the rotation planes were expressed in spherical coordinates (θ, ϕ). In this notation, the main directions of the reference frame are (90°, 0°) for *a*^{*}, (90°, 90°) for *b* and (0°, 0°) for *c*.

Analytic grade (>98%) sucrose was purchased from Sigma-Aldrich and used without further purification. Sufficiently large crystals were carefully selected and visually oriented to allow rotation of the static magnetic field around the main directions of the reference frame. The crystals were then polished to fill 1.4 mm internal diameter quartz tubes and irradiated using a Philips tungsten anode X-ray tube operated at 60 kV and 40 mA at RT for at least 3 h, which amounts to a >100 kGy dose. Exact rotation planes were later determined by fitting angular variations of ENDOR lines ascribed to the largest couplings of the known stable radicals: H_{β1} of T1, H_α of T2 and H_α of T3 HF tensors from Ref. 15. EPR spectra were recorded at RT and 110 K in X (9.5 GHz), Q (34 GHz) and W (94 GHz) microwave frequency bands using 12.5 kHz modulation frequency and 0.1 mT modulation amplitude. ENDOR and EIE spectra have been recorded in Q-band at 110 K using 200 μ W microwave power, ca. 2.5 W radio frequency (RF) power and 350 kHz modulation depth. Further details of the employed setups and experimental parameters are summarized in Table 1.

Fittings of angular variations and simulations of spectra throughout this work were performed with MatlabTM using EasySpin routines¹⁹, based on numerical matrix diagonalization

of the spin Hamiltonian:

$$\hat{H}_S = \mu_B \vec{B} \hat{g} \vec{S} + \sum_k \left(\hat{S} \hat{A}_k \hat{I}_k - g_N \vec{B} \hat{I}_k \right),$$

in which symbols have their usual meaning and *g_N* refers to the proton nuclear *g* factor. An isotropic **g** tensor was assumed for simulations of ENDOR roadmaps. Simulation parameters for the known stable radicals T1–T3 were taken from Ref. 15. DFT calculations were performed using the B3LYP hybrid functional^{20,21}, specialized triple- ζ (EPR-III) basis sets²² and in the spin-orbit mean field approach²³, as implemented in the ORCA package^{24,25}. These settings were chosen relying on our previous periodic studies^{15,26,27}. The resulting molecular fragment was visualized with VMD software²⁸.

3 Results and Discussion

3.1 Single Crystal EPR-ENDOR-EIE Analysis at 110 K

As is often the case for organic radicals, the spectra of all of the stable species in X-irradiated sucrose largely overlap because of a similar weak *g* anisotropy and a large number of HF interactions. Representative Q-band spectra at the main directions of the reference frame, recorded at 110 K, are presented in Figure 1A. It has been shown that the central part of this spectrum can be well explained by considering contributions of the three known stable radicals¹⁵. Features in the wings of the spectrum remained unexplained and, as it will be shown further, can now be accounted for by including a fourth stable species (T4) in the analysis.

For most magnetic field orientations, a field range can be found where the EPR spectrum of T4 does not overlap with those of T1–T3. We exploited this to record ENDOR spectra with minimal interference of the other stable radicals. Additionally the resonance line ascribed to the largest HF coupling (HF1) in T4's ENDOR spectrum is well isolated from the other lines. This in turn permitted to measure EIE spectra of T4 (Figure 1B), undisturbed by other stable species. The spectra of T4 at all main orientations can be attributed to a radical with an unpaired electron (*S* = 1/2) coupled to four protons (*I* = 1/2) by HF interactions of diminishing strengths, that is HF1 > HF2 > HF3 > HF4. At *a*^{*} and *c* the spectra have a structure of a doublet of broad triplets and a doublet of quintuplets, respectively. At *b* it takes shape of a quadruplet of doublets of equal intensities, indicating that HF4 $\approx$ 0 at that orientation. Indeed, in the ENDOR spectrum with *B* || *b* (Figure 1C) splitting HF4 becomes very small and its transitions lie very close to the ¹H Larmor frequency.

The single crystal analysis was performed by following an established and validated methodology^{15,29}. Roadmaps of ENDOR lines ascribed to HF1–HF4 were constructed by following the resonance positions in four rotation planes (Figure 2A). At some orientations, ENDOR spectra had to be recorded at both low- and high-field regions of the spectrum, to obtain data points for both symmetry related sites. Angular variations of the EIE pattern centers (Figure 2B) were used to determine the **g** tensor. The root-mean-square error per point (RMSE) for the four determined HF tensors is RMSE = 0.01 MHz, while the correlation coefficient *R*² = 0.9829. For the **g** tensor, *R*² = 0.9829 and RMSE = 0.0002. The

Table 1 Overview of the experimental details of the setups used in the present work.

	X	Q	W
Spectrometer	Bruker ESP300E	Bruker ElexSys E500	Bruker ElexSys E680
Field measurement	Bruker ER 035M	Bruker ER 035M	internal
Field calibration	DPPH ($g = 2.0036$)	$g_{\perp} = 2.0031$ of CO_3^{3-} in irradiated CaCO_3 ¹⁷	Mn^{2+} in MnO ¹⁸
Frequency counter	HP5350 B	Pendulum CNT-90XL	internal in the bridge
μW power	10 μW @ 110 K and RT	10 μW @ 110 K and RT	50 nW @ 110 K, 10 μW @ RT
Cryostat	Oxford ESR900	Oxford CF935	Oxford CF935

errors on the principal values were determined using MAGRES ($\Delta g = 0.0002$)³⁰. The accuracy of the principal g values has been further improved to $\Delta g \approx 0.00005$ with the aid of powder simulations of W-band spectra, which are discussed in Subsection 3.4. All determined tensors are presented in Table 2.

It is important to note that with the available data set it was possible to solve the usually arising ambiguities for the experimental tensors. The Schonland ambiguity^{31,32}, that stems from a freedom in choosing the rotation sense in all of the rotation planes, was solved by recording spectra in a fourth skewed rotation plane. Simulations of angular variations of the EIE spectra (Figure S1 in the Supporting Information) permitted the consistent assignment of the symmetry sites of all determined tensors, which is crucial for accurate powder simulations⁴. The signs of the principal HF values were determined relying on previous experience and physical intuition^{12,13,33}, and confirmed by DFT calculations (Subsection 3.3).

3.2 Powder ENDOR and Method for Isolating the Powder EPR Spectrum of T4

In order to check whether the T4 radical indeed is the as yet unidentified contribution to the dosimetric EPR spectrum of sucrose, ENDOR spectra were also recorded on irradiated sucrose powders as a function of static magnetic field position. The high frequency part of this Field-Frequency ENDOR (FF-ENDOR) spectrum is shown in Figure 3B. As a result of the orientation selectivity of ENDOR³⁴, this spectrum reveals the anisotropy of the ^1H HF interaction and of the g tensor. The simulation (Figure 3C), using the spin Hamiltonian parameters for T4 in Table 2, as determined from the single crystal analysis, reproduces the experimental FF-ENDOR spectrum very well. This also holds true for single traces, as illustrated in Figure 3A.

For single crystals, the contribution to the EPR spectrum of a radical with $S = 1/2$ can be isolated out of a composite EPR spectrum by recording the EIE spectrum on an ENDOR transition for a nucleus with $I = 1/2$, as is illustrated in Figure 1 and in supporting Figure S1. EIE relies on the fact that for $I = 1/2$ nuclei interacting with an $S = 1/2$ electron system, the signal height of an ENDOR transition is in a (rather) good approximation proportional to the EPR signal strength³⁵. This principle can, however, not be directly applied to reconstruct the powder EPR spectrum of a radical contribution responsible for a particular transition in powder ENDOR spectra. A generalization of the idea behind EIE is needed.

The contribution of interaction HF1 to the powder ENDOR spectra is well-isolated from all other contributions (see Figure 3B). For such interactions, one may expect the total integrated intensity of the ENDOR spectrum to be proportional to the signal strength of the EPR spectrum of the radical, responsible for this HF interaction. One obtains this total integrated intensity by integrating the horizontal traces in the frequency modulated FF-ENDOR spectrum one time to obtain absorption-like ENDOR spectra and a second time to obtain the total intensity of the absorption spectra. The result of such "double integration" of the ENDOR spectra, as a function of magnetic field position is shown in Figure 3D for the experimental (blue curve) and for the simulated patterns (green curve). These EIE-like spectra are also compared with the absorption powder EPR spectrum simulated for the T4 radical using the spin Hamiltonian parameters in Table 2. These three spectra are in very good agreement with one another, indicating that this technique indeed yields the generalization of EIE spectroscopy we are looking for. Obviously, this technique only leads to completely isolated powder EPR contributions for HF interactions which exhibit no overlap in the powder ENDOR spectra. If these conditions are met, the technique should be effective even if relaxation properties of spectral components are identical and echo-detected EPR decomposition proves to be challenging³⁶. Even if the ENDOR components are not completely separated, on the other hand, it may still yield partial decomposition of complex powder EPR spectra.

Figure 4 compares the EIE-like spectrum of T4 to the integrated powder EPR spectrum of irradiated sucrose at 110 K. Also shown in this figure are the simulated absorption powder EPR spectra of all four stable radical contributions individually, and a weighed sum of these that matches the experimental powder spectrum best. A more detailed comparison in first-derivative mode is presented and discussed in Subsection 3.4. At this moment several points may be noted.

1. The four stable radical contributions that have been characterized so far reproduce the total powder spectrum completely: there is no need to assume that other radicals contribute in a significant way.
2. Although the contribution of T4 to the total EPR intensity is considerable (ca. 15% see Subsection 3.4) its EPR amplitude at any magnetic field position is small in comparison to the total EPR amplitude in the center of the pattern. In spite of this, we succeeded to isolate this contribution with the EIE-like technique described above.

Table 2 Experimentally determined (at 110 K) **g** and HF tensors of the fourth stable radical species in X-irradiated sucrose.

Tensor	<i>g</i> -value	δg^a	Iso ^b	Aniso ^b	Eigenvectors		
					<i>a</i> [*]	<i>b</i>	<i>c</i>
g	2.00206	-0.26			0.369	0.080	0.926
	2.00439	2.07			0.510	-0.851	-0.130
	2.00646	4.14			0.777	0.520	-0.355
HF1 (H _{β1})			80.12	-4.31	-0.198	0.721	0.664
				-1.45	-0.918	0.102	-0.384
				5.76	-0.344	-0.685	0.642
HF2 (H _{β2})			36.22	-3.84	0.432	-0.279	0.858
				-2.42	-0.794	0.334	0.508
				6.26	-0.428	-0.901	-0.077
HF3 (H _{β3})			-15.78	-8.09	0.757	0.582	-0.296
				-3.07	0.304	0.086	0.949
				11.17	0.578	-0.809	-0.112
HF4 (H _{HO})			-7.07	-7.96	0.419	0.011	0.908
				-6.56	0.820	-0.434	-0.373
				14.52	0.389	0.901	-0.191

a *g*-values are presented as shifts from the free electron *g*-value $g_e = 2.002319$ and expressed in ppt.

b Isotropic and anisotropic values of the HF tensors are expressed in MHz.

3. The EIE-like spectrum of T4 is characterized by two large HF interactions. Next to HF1, the interaction observed with FF-ENDOR (ca. 80 MHz splitting in the EPR spectrum), a second splitting is resolved (ca. 36 MHz), corresponding to HF2. This interaction is difficult to recognize in the ENDOR spectra, since its ENDOR transitions are overlapping with those of interactions of similar strength for the other stable radicals. Moreover, already in the single crystal spectra the ENDOR transitions of HF2 for T4 are considerably less intense than for HF1. Like EIE, the technique we introduced here for powders may provide information on HF interactions that are difficult to extract directly from the ENDOR spectra.

The discussion above indicates that this technique for reconstruction of the powder EPR spectrum from FF-ENDOR spectra may prove very useful in practical cases where no single crystal data are available, like for DNA. It may also be viewed as complementary to echo-detected EPR experiments.

3.3 Chemical Structure of T4

The **g** tensor of T4 suggests that it is a C-centered radical with a carbonyl group in a β -position. Comparing its anisotropy to that of the **g** tensors for T1-T3 (Table 3), a closer resemblance is found to the allylic-type radical T1, than to T2 or T3¹⁵. The two largest HF tensors of T4 (HF1, HF2) have isotropic values and anisotropic features typical for β -¹H interactions, the third one (HF3) can be identified as a β -¹H adjacent to the carbonyl group, also observed in T1¹⁴, and the smallest interaction (HF4) arises from a β -hydroxyl proton. From this it follows that another apparent similarity between T1 and T4 is the lack of an α -¹H coupling,

which is present both in T2 and T3¹³. It can immediately be seen that both β -H atoms have to be located on the same carbon by counting the chemical bonds (Figure 5A). The C atom bearing most of the spin density makes one bond with the hydroxyl group, then another bond has to be made with the formyl group, leaving only one possible C position for both remaining H atoms. The resulting chemical structure was verified using *in vacuo* DFT calculations on an isolated molecular fragment with a -CH₂-OH terminator.

The optimized structure, with the C2"-O2"-HO2" bond angle constrained to 90° and the O2"-C2"-C3"-H3"a dihedral angle — to 72°, is shown in Figure 5B. Calculated tensors (Table 4) of the four largest HF interactions are presented and compared to the corresponding experimental tensors (Table 2), while interactions with the ¹H nuclei of the terminator were so small (<1 MHz) that they could be neglected. The experimental and calculated HF tensors were compared in the reference frames of the corresponding **g** tensors, because the experimental parameters are fixed in the $\langle a^*bc \rangle$ frame, whereas the calculation reference frame is arbitrary. Experimental HF tensors, expressed in the reference frame of the **g** tensor, are available in Table S2 in the Supporting Information. The agreement of the eigenvalues and principal directions is remarkably good. The *A_x* and *A_y* directions of the second largest HF interaction and the isotropic value of the largest one seem to deviate slightly more than other values, but this is reasonable for the small number of constraints applied. The calculated *g* values are slightly overestimated, which is a known feature of DFT¹⁵, but the overall anisotropy is reproduced rather well. This good correspondence also shows that site and sign ambiguities were solved correctly.

To accommodate all these features, severe alterations have to

Table 3 Comparison of principal values of the experimental **g** tensors suggests that T4 is more similar to T1 than to T2 and T3. *g*-values are presented as shifts from the free electron *g*-value $g_e = 2.002319$ and expressed in ppt.

Radical	δg_1	δg_2	δg_3
T1 ^a	-0.2	2.6	4.3
T2 ^a	-0.3	3.1	4.4
T3 ^a	-0.3	3.2	4.4
T4 ^b	-0.3	2.1	4.1

^a Ref. 15.

^b Present work.

Table 4 Calculated **g** and HF tensors of a molecular fragment depicted in Figure 5C. Deviation angles δ are defined as angles between eigenvectors of the calculated tensors and corresponding vectors of the experimental tensors expressed in the reference frame of the **g** tensor (Table S2 in the Supporting Information). HF interaction tensors are compared in the following way: H3"^a — to H_{β1}, H3"^b — to H_{β2}, H1" — to H_{β3} and H2" — to H_{HO}.

Tensor	<i>g</i> -value	δg^a	Iso ^b	Aniso ^b	Eigenvectors			δ^c
					<i>x</i>	<i>y</i>	<i>z</i>	
g	2.00217	-0.15			1.000	0.000	0.000	
	2.00529	2.97			0.000	1.000	0.000	
	2.00720	4.89			0.000	0.000	1.000	
H3" ^a			67.35	-3.50	0.591	-0.806	0.027	2
				-2.71	-0.701	-0.530	-0.476	3
				6.21	0.399	0.262	-0.879	4
H3" ^b			38.03	-3.56	0.964	0.006	-0.265	22
				-2.38	-0.111	-0.899	-0.424	23
				5.94	-0.241	0.438	-0.866	9
H1"			-13.02	-6.71	0.003	-0.142	0.990	5
				-3.52	1.000	-0.002	-0.003	4
				10.23	0.002	0.990	0.142	5
H2"			-9.61	-10.74	1.000	-0.008	-0.006	5
				-3.00	0.010	0.834	0.552	5
				13.74	0.001	-0.552	0.834	2

^a *g*-values are presented as shifts from the free electron *g*-value $g_e = 2.002319$ and expressed in ppt.

^b Isotropic and anisotropic values of the HF tensors are expressed in MHz.

^c Deviation angles are expressed in degrees.

happen in the sucrose molecule. The formation of a carbonyl group will not be possible if only simple models, like single H-abstractions, are considered. Multiple H-abstractions and possibly cleavage of the glycosidic bond or rupture of a ring in (at least) one of the sugar units should also be taken into account. Broken glycosidic bonds are quite commonly found in stable radicals in sucrose^{12–14}. Ruptured rings, on the other hand, are very uncommon in disaccharides, but there are no obvious obstacles for them to occur and they were reported for fructose³⁷. In a sucrose molecule, two H atoms on a single carbon can only be found at the C6 and C6' sites, (for atom labeling see Figure 5C,) making C5 and C5' the most likely candidates for the radical center. Comparisons to directions in a pristine molecule were performed, but not even marginal resemblance can be reported. At the moment, we have a very good understanding of the radical's chemical structure, but its embedding in the crystal lattice remains unknown. An exhaustive DFT study, that goes beyond the

scope of this work, would have to be performed to determine the detailed lattice environment of the T4 radical.

3.4 Powder EPR Spectrum Simulations

From the viewpoint of dosimetry, reproduction of the RT X-band EPR spectrum of irradiated sucrose is the most relevant. Spectra recorded at higher microwave frequencies are obviously also interesting for determining the radical composition. In this study we included also the Q- and W-band powder EPR spectra. Since the spin Hamiltonian parameters of the T1–T4 radicals have been determined at 110 K and temperature dependence of these parameters cannot be excluded *a priori*, the 110 K powder spectra at these three microwave frequencies are included in the comparison as well. These experimental spectra, simulations for individual radical components and best fit linear combinations of these simulated spectra are summarized in Figure 6.

As explained in detail by De Cooman et al.¹⁵, anisotropic EPR

linewidth parameters for four spectral components and three relative weight factors implies 27 fitting parameters for a single powder EPR spectrum. This number is increased further when fitting multiple spectra, considering that line widths may be microwave frequency and temperature dependent. A thorough investigation of the influence of all these free parameters is beyond the scope of this paper. For this reason, we have severely restricted the degrees of freedom in the simulations. We have taken as a fixed starting point the linewidth parameters and relative contributions of T1–T3, as determined from a limited fitting procedure of the central part of the powder spectra, excluding the contributions of T4, as presented in Ref. 15. The EPR linewidth parameters of T4 in each powder spectrum are chosen proportional to the parameters determined from the single crystal EIE analysis. Together with the weight factor of T4 in all spectra, this adds up to 4 parameters for fitting the 3 experimental spectra recorded at 110 K.

The final relative contributions of all four stable radicals were found as 0.38:0.28:0.19:0.15 for T1:T2:T3:T4, respectively. It is worth noting that the contribution of T4 (ca. 15%) is larger than expected during previous studies. The largest features of the fourth species in X- and Q-bands are in the region where three other stable radicals are dominant, explaining why the uncertainties of the previous estimates were quite large. All resolved features in the experimental spectra are reproduced by the simulations, and in the 110 K spectra they appear in the correct field positions. The relative intensities of the lines are not perfectly reproduced, but taking into account the very limited number of parameters fitted, the agreement between simulations and the 110 K spectra may be considered as very good.

Comparing the RT and 110 K experimental spectra in X- and Q-band, slight differences are observed. Most noteworthy are the considerable shifts in the positions of the wing features, solely due to T4. In the W-band spectra certain features in the middle part have also shifted in position. Comparison with the simulated single components indicates that these features belong to T4. This strongly indicates that the spin Hamiltonian parameters of T4 exhibit a more pronounced temperature dependence than those of T1–T3. Indeed, only a very subtle change in the HF parameters of T1–T3 was found in our previous studies¹². The changes between 110 K and RT spectra are not indicative of a change in the number of spectral components. Nonetheless, the temperature dependence of the T4 component will have to be accounted for in future decomposition studies of the dosimetric EPR spectrum of irradiated sucrose.

4 Conclusions

A fourth stable radical species in X-irradiated sucrose has been characterized using ENDOR and EIE spectroscopy. Four ¹H HF tensors and the g tensor were determined from a Q-band single-crystal analysis. The experimental spin Hamiltonian parameters suggest that this fourth radical species is C-centered, with two H atoms on the same carbon, a carbonyl group and a hydroxyl group in β-positions. We were not able to determine the exact conformation of the radical in the crystal lattice, considering that severe alterations to the pristine sucrose lattice have to occur in order to accommodate all of the aforementioned chem-

ical features. It is probable, however, that the radical is centered on C5 or C5', since these are the only locations in the molecule where two β-H atoms can naturally be found on one of the neighboring carbons — C6 and C6', respectively. An EPR powder study at 110 K and RT in three frequency bands (X, Q and W) revealed that the composite nature of the EPR spectrum of X-irradiated sucrose can now be completely understood. In comparison to other known stable radicals, the spectrum of the fourth species displays a more pronounced, but still small, temperature dependence.

The ENDOR spectrum of the largest HF coupling is observed in the ENDOR spectra above 90 MHz, where there is no interference with resonance lines of other HF interactions. We exploited this peculiarity to extract an absorption-like EPR pattern of T4 by integrating a segment of the full FF-ENDOR spectrum over the RF range. This technique can be viewed as a generalization of the well-established EIE experiments, applicable to disordered systems. This approach has the potential to be useful for decomposing multicomponent EPR spectra of systems where single-crystal samples are not available and where some of the contributing species exhibit large HF interactions, e.g. radiation-induced sugar radicals in DNA.

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Fig. 1 Stable (A) EPR, (B) EIE and (C) ENDOR spectra of X-irradiated single-crystal sucrose at orientations close to the main directions of the reference frame were recorded at 110 K. Experimental EPR and EIE spectra (black lines) agree rather well with the simulations (blue lines). Simulations of T1-T3 were performed using simulation parameters from Ref. 15 and of T4 — with the experimental spin Hamiltonian parameters (Table 2) and anisotropic line broadenings of (16, 10, 13) MHz. Intensity ratios of 0.38:0.28:0.19:0.15 for T1:T2:T3:T4 were determined from the analysis of EPR powder patterns (Figure 6). Black arrows over the EPR spectra mark static field positions, where ENDOR spectra were recorded. In the ENDOR spectra, an artifact line of our setup at 88.6 MHz is indicated by an asterisk (*).

Fig. 2 Angular variations of T4's ENDOR lines ascribed to interactions HF1–HF4 (A) and g -values determined from EIE spectra (B) in four rotation planes were recorded at 110 K. ENDOR spectra were normalized to a ^1H Larmor frequency of 51.5 MHz and EIE spectra — to a microwave frequency of 34 GHz. Simulations of resonance roadmaps (black lines) were performed using experimental data from Table 2. In the fourth (skewed) rotation plane, simulations of the Schonland conjugates (Table S1 in the Supporting Information) are presented in red lines. Lines ascribed to HF3 in the fourth plane are depicted in dashed lines for better readability. Unit vectors defining rotation planes expressed in spherical coordinates are $(92^\circ, 1^\circ)$ for $\text{rot}\langle a^* \rangle$, $(90^\circ, 90^\circ)$ for $\text{rot}\langle b \rangle$, $(3^\circ, 94^\circ)$ for $\text{rot}\langle c \rangle$ and $(-54^\circ, 95^\circ)$ for the fourth plane.

Fig. 3 (A) Experimental (black line) ENDOR spectra of the largest HF interaction of T4 in X-irradiated sucrose powder were recorded and compared to simulated ones (purple line). ENDOR simulations were performed using spin Hamiltonian parameters determined from single crystal analysis (Table 2), Gaussian line broadenings of 0.75 MHz and 0.5 mT excitation width. Considering the limitations of the linewidth model used, the agreement for individual spectra is very good. The experimental (B) and simulated (C) FF-ENDOR spectra, presented as color scale intensity plots (red — high, blue — low), agree very well. (D) Finally, the simulated EPR absorption spectrum of T4 (red line) is compared to experimental (blue line) and simulated (green line) FF-ENDOR spectra, integrated twice over the RF frequency.

Fig. 4 (A) Decomposition of the integrated Q-band EPR spectrum (black solid line) of X-ray irradiated sucrose powder at 110 K. The sum spectrum of stable radicals (blue line) consists of four components (T1–T3 — light grey dashed lines, T4 — dark grey dashed line), which are scaled here by appropriate weights, determined from an analysis of powder EPR spectra (Figure 6). (B) Comparison of the integrated FF-ENDOR spectrum of the largest HF coupling of T4 (red line) to the simulated absorption spectrum using single crystal data (Table 2) reveals that the spectra are structurally the same, despite some differences in relative intensities of the main peaks.

Fig. 5 (A) Chemical structure of the fourth stable radical species. (B) Optimized structure of a molecular fragment, that has all chemical features of the fourth stable radical, and the atom labeling scheme. C atoms are depicted in cyan, O — red, H — white. Carbons are numbered as shown above, carbon-bound hydrogen and oxygen atoms are labeled according to the carbon to which they are bound and hydroxyl hydrogen atoms — according to the oxygen to which they are bound. (C) Chemical structure of a pristine sucrose molecule. Atoms are numbered in the same way as for the molecular fragment.

Fig. 6 Comparison of simulated EPR spectra of stable radicals (black lines) and their sums (blue lines) to experimental spectra (red lines) of X-ray irradiated sucrose powder, recorded at 110 K and RT in (A) X-, (B) Q- and (C) W-bands. Simulation parameters of T1–T3 were taken from Ref. 15, while the ones of T4 were determined from a single crystal EIE analysis (Table 2 and Figure 1). Weight ratios of 0.38:0.28:0.19:0.15 were determined by a non-linear χ^2 fit using X- and Q-band spectra. W-band spectra allowed us to determine the principal values of the T4's $\mathbf{g}$ tensor with higher precision. Line width proportionality factors of 0.7, 0.9 and 1 were used for simulations of spectra, recorded at X-, Q- and W-band, respectively.