Radiation Damage in Carnidazole: a Single Crystal EPR Study

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Abstract. Single crystals of hydrated and anhydrous carnidazole have been subjected to X-irradiation at 77 K and studied by EPR. The determination of the EPR tensors shows that NO\textsubscript{2}, sulfur-centered radicals as well as a radical pair can be generated from this molecule; it appears that the species trapped at 77 K are not the same in the hydrated and in the anhydrous crystal. The results are discussed in the context of the radiosensitizing action of molecules containing a 5-nitroimidazole moiety.

Introduction

Nitroimidazole compounds are intensively studied in radiation biology because of their potential use in radiotherapy [1], and metronidazole, e.g., has been shown to be an effective radiosensitizer of hypoxic cells [2]. However, the molecular mechanism of such a radiosensitization process is still poorly understood, and more information is needed concerning the nature of the radicals resulting from exposure of these compounds to ionizing radiation. By studying the radiation damage in crystalline O-methyl [2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl]thiocarbamate (carnidazole, Fig. 1), we show that the radiation behaviour of nitroimidazole compounds is not only determined by their molecular structure but is very dependent upon their environment. For this purpose, we will analyse the EPR spectra due to radiogenic radicals trapped in two slightly different matrices corresponding, respectively, to a hydrated and an anhydrous carnidazole single crystal.

Experimental

X-rays diffraction showed us that two types of crystals can be simultaneously obtained by slow evaporation of a soln. of carnidazole in a H\textsubscript{2}O/EtOH mixture: 1) crystals having the same parameters as those previously studied by Blaton et al. [3] and identified as being crystals of carnidazole·H\textsubscript{2}O, 2) crystals whose structure has been determined [4] in our laboratories, and which appear to be anh. crystals of carnidazole.

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Results

An ORTEP illustration of the carnidazole molecule, obtained from the crystal structure of the anhydrous compound [4], is shown in Fig. 1. Although the unit cell parameters are different, the molecular structure is very similar with that determined from the hydrated crystal; the main conformational difference lies in the torsion angle N(1)-C(4)-C(5)-N(4) which is equal to 175.0° in the anhydrous crystal and to 60.6° in the hydrated crystal.

Hydrated Crystal of Carnidazole

We show, in Fig. 2, an example of an EPR spectrum obtained at 77 K with a single crystal of hydrated carnidazole subjected to X-irradiation under liquid N\textsubscript{2}. Signals marked A are due to a species exhibiting coupling with a spin-1 nucleus, whereas no hyperfine structure can be observed for the signal marked B. Signals

Fig. 1. ORTEP plot of the carnidazole molecule (anhydrous crystal)

Fig. 2. Example of an EPR spectrum, obtained at 77 K, with an X-irradiated single crystal of hydrated carnidazole
marked C are more difficult to analyze, because, for several orientations of the magnetic field, their hyperfine structure is hidden by overlap with signals due to other species. Nevertheless, the angular variation of this four-line pattern is consistent with hyperfine coupling with both a spin-1 and a spin-1/2 nucleus, and we have analyzed these signals by assuming an interaction with a \(^{14}\)N and a \(^{1}\)H nucleus. A variable-temperature experiment showed that signals B and C decay at 120 K, whereas signals A are still stable up to 240 K. The angular variation of the various signals lead to the tensors shown in Table 1.

### Anhydrous Crystal of Carnidazole

An example of an EPR spectrum obtained at 77 K with a single crystal of anhydrous carinedazole is shown in Fig. 3. The angular variation of the signals marked A’ and B’ could be followed in the three reference planes and will be discussed here. The strong anisotropy of the signals A’ suggested that they were due to a radical pair, and this hypothesis was confirmed by observing the forbidden half-field signal (\(\Delta M = 2\) transition). The angular variation of the signals A was, therefore, analyzed by using a Hamiltonian which takes the electronic Zeeman effect and the electron-electron dipolar interaction (\(D\) tensor) into account. The signal B’ does not exhibit any structure and can be characterized only by its g tensor. The various EPR tensors obtained from the anhydrous crystal are shown in Table 2.

### Discussion

The hyperfine tensor reported for the radical A trapped in the hydrated crystal is shown in Table 1 together with the tensor obtained by Eda and Iwasaki for \(\text{NO}_2\) trapped in diglycine nitrate [7]. It appears clearly that the species A can be identified as being the \(\text{NO}_2\) radical produced by homolytic scission of a C–N bond. The radical B, trapped in the same crystal, is characterized by a very large anisotropy of the g factor which is similar with that observed for RS radicals [8]. This species probably results from the addition of a radiogenic radical (e.g. \(\text{NO}_2\) structure r1) to the C=S bond. As shown in Table 1, the g tensor of the radical C is also very anisotropic, and indicates that the corresponding species is also a S-centred radical. However, the hyperfine interaction with both a nitrogen and a proton nucleus suggests for C either a more delocalized structure (like r2) or the addition, on the C=S bond, of an H-atom (formed by radiolysis of \(\text{H}_2\text{O}\)) leading to hyperfine coupling through hyperconjugation. Such a species could be represented by the structure r3 which assumes that the torsion angle between the axis of the sulfur-p orbital containing the unpaired electron and the C=O bond direction is more suitable for hyperconjugation than in the case of the structure r1. The appreciable anisotropy of the hyperfine tensors measured for C, however, makes the structure r2 more plausible.

The paramagnetic species A’ trapped in the anhydrous crystal is a pair formed by two radicals separated by a distance r.

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**Table 1. EPR Tensors for the Various Radical Species Trapped in an X-Irradiated Single Crystal of Hydrated Carnidazole (A, B, C).** The values for \(\text{NO}_2\) have been obtained by Eda and Iwasaki from diglycine nitrate [7].

| Radical A | g = 1.991 | g = 2.001 | g = 2.004 |
|-----------|-----------|-----------|-----------|
| \(^{14}\)N-T [MHz] | T = 136 | T = 159 | T = 188 |
| g = 0.678 | 0.680 | 0.627 |
| \(^{1}\)H-T [MHz] | T = 55 | 0.376 | 0.757 |

**Table 2. EPR Tensors for \(\text{NO}_2\).**

| g = 1.9907 | g = 2.0018 | g = 2.004 |
|-----------|-----------|-----------|
| \(^{14}\)N-T [MHz] | T = 136 | 0.027 | 0.027 |
| 0.516 | -0.523 | 0.677 |
which can be estimated from the $D$ tensor: the axiality of this tensor makes use of the point dipole approximation applicable and $D_{||}$ shows that the $r$ distance is about 5.4 Å. The eigenvector $D_{||}$ is expected to be aligned along the $r$ vector and comparison with the crystal structure of the precursor shows that $D_{||}$ makes an angle of $5^\circ$ with the vector linking the thiocarboxylate oxygen of two adjacent molecules in the unit cell. This result suggests, therefore, that the two components of the pair are two RC(S)O radicals (structure r4) which result from homolytic scission of O–CH$_3$ bonds and that the two Me fragments recombine to form an ethane molecule. This interpretation is consistent with the $g$ tensor whose eigenvalues are similar with those previously reported for alkoxyl radicals and whose $g_{\text{max}}$ eigenvector is, as expected, close to the C–O direction (C–O, $g_{\text{max}} = 2.0$). It is worth mentioning that this process requires that the distance between the two MeO groups be relatively short in the precursor; this distance is 5.37 Å in the anhydrous crystal (where the pair is indeed observed), whereas it is 6.5 Å in the hydrated crystal (where the pair is not observed). The radical B', which is the second species trapped in the anhydrous crystal, has a $g$ tensor similar to that measured for the radical B in the hydrated crystal and is also a S-centered radical which has probably a structure of the type r1. To summarize, two sorts of radiation damage are observed in carmidazole crystals: the first is due to the formation and reactivity of NO$_2$ radicals, the second is independent of the S-nitroimidazole moiety.

Many mechanisms of radiosensitization by compounds containing a nitroimidazole moiety have been proposed in the literature [2a]. E.g., these compounds have been suggested to react with radiation-induced free radicals in vital macromolecules and to prevent the repairing of these molecules by H-atoms formed from sulfhydryl groups. It was also proposed that the high electron affinity of nitroimidazole compounds indirectly increases the extent of cationic damage in DNA. In these mechanisms the radiosensitization is thought to result from the properties of the whole nitroheterocycle. However, a previous study [9] on radiogenic radicals produced from a heterocycle containing a NO$_2$ group showed that the NO$_2$ radical was easily produced and could lead to secondary radicals. In carmidazole, the NO$_2$ radical is also produced, since it could be trapped in the hydrated crystal. It could not be stabilized in the lattice of the anhydrous compound, but with both crystals, however, a species was formed (radical B and B’) which may be due to the addition of a NO$_2$ radical on a C-atom of the thio-

### Table 2. EPR Tensors Measured for Two Radicals (A’ and B’) Trapped, at 77 K, in an X-Irradiated Single Crystal of Hydrated Carnidazole

| Eigenvectors | Eigenvalues |
|--------------|-------------|
| $/X$         | $g_{A'}$= 2.0035, 0.576, 0.431, 0.694 |
| $/Y$         | $g_{B'}$= 2.0076, 0.223, -0.900, 0.373 |
| $/Z$         | $g_{B'}$= 2.0128, 0.786, -0.059, -0.615 |

### Fig. 3. Example of an EPR spectrum, obtained at 77 K, with an X-irradiated single crystal of anhydrous carmidazole

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[9] Crystal structure determination: C$_6$H$_{12}$NaO$_2$S, $M_t$ = 244.3, monoclinic, $P2_1/a$, $a = 7.304$(1), $b = 15.437$(3), $c = 10.650$(4) Å, $\beta = 109.47$(1)*. $\gamma = 113.2.(5)$Å$^2$, $Z = 4$, $a_0 = 1.43$ g cm$^{-3}$, $\mu = 0.272$ mm$^{-1}$, $\text{Fo}$ = 512, 1477 measured reflections at room temperature (Philips PW1100 diffractometer), 890 observed ($I_{F_{o}} > 4I_{F_{c}}$), $R = 0.067$, $\alpha_{o} = 0.032$ ($\omega = 1/2$, $\phi_{max}$) 181 variables with non-H-atoms refined with anisotropic displacement parameters. All coordinates of the H-atoms have been observed and refined. Full details of the crystal structure are submitted for publication (G. Bernardinelli, T. Berclaz, M. Geoffroy, N. Rajalakshmi, *Acta Crystallogr.*, *C*).

For the hydrated crystal of carmidazole, the coordinates of the unit cell vectors $a$, $b$, $c$ in the cartesian EPR reference frame XYZ are respectively: $a = 23$, $0.02$, $-6.47$ Å; $b = 8.50$, $-18.56$, $0.09$ Å; $c = -8.35$, $-3.33$, $3.82$ Å. For the anhydrous crystal, the EPR reference axes are defined as follows: $Y$ is aligned along $-b$, $X$ makes an angle of $40^\circ$ and $110.5^\circ$ with $a^*$ and $c^*$, respectively.

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