

## COMMENTS

## Comment on “Effects of Ionizing Radiation on Crystalline Cytosine Monohydrate”

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Received: October 27, 1998

In their recent paper, Wetmore et al.<sup>1</sup> report DFT calculations on oxidation and reduction products observed in irradiated single crystals of cytosine monohydrate. The theoretical results are compared with experimental EPR/ENDOR results, mainly those of Sagstuen et al.,<sup>2</sup> and the calculations generally agree nicely with hyperfine couplings derived from the experimental data.

However, Wetmore et al.<sup>1</sup> repeatedly recommend reinvestigation of radical assignments when the agreement is poor. Unfortunately, they seem to overlook the possibility that the calculations may be less realistic than the experimental results. One important point, emphasized by the authors, is that these detailed DFT calculations were performed on isolated molecules, whereas the experimental results are from radicals formed in the solid state, mainly in single crystals. The DFT calculations omit the electrostatic environment of the radicals, particularly the intricate hydrogen bonding structure in which the free radicals are imbedded.

In weighing theoretical vs experimental results, Wetmore et al.<sup>1</sup> overlook the strengths of the actual EPR/ENDOR measurements. In the experiments, single crystals were accurately oriented with an X-ray precession camera, X-ray irradiated, and observed at ca. 10 K. Anisotropic hyperfine couplings were measured by rotating the crystals in the external magnetic field. From hundreds of angular measurements, various proton and nitrogen hyperfine couplings were obtained. Direction cosines associated with each coupling were compared with specific molecular directions known from the X-ray crystal structure, and the major sites of unpaired spin density were identified.

All this information permits establishment of consistent models for the molecular structure of the primary oxidation and reduction products in the irradiated crystals. This is usually based on the precise information about major sites of spin density. However, assignment of the numerous small hyperfine couplings found with ENDOR data is difficult; thus, establishing the precise protonation state of a model may be difficult. To solve this problem, one can repeat the entire experiment with partially deuterated crystals to learn which of the many small hyperfine

couplings are due to exchangeable protons. In their analysis Wetmore et al.<sup>1</sup> also seem to miss this point.

Wetmore et al.<sup>1</sup> discuss oxidation of the cytosine base. Sagstuen et al.<sup>2</sup> assigned the primary oxidation radical as the N1 deprotonated cation. ENDOR experiments show that approximately 57% of the unpaired spin density is on C5 and that two small exchangeable couplings have angular variations corresponding to the N4–H protons. Finally, about 30% spin density was assigned to N1. From DFT calculations on four different deprotonated cations, Wetmore et al.<sup>1</sup> say that the N1 deprotonated cation best fits the experimental results (C5: 49%, N1: 29%, N4: negligible). However, since their calculated C5–H isotropic hyperfine coupling (–31.5 MHz) is significantly different from the experimental value (–41.5 MHz), and their calculation predicts only small N4 spin density, they reject the N1 deprotonated cation model.

The N1 deprotonated cation is the best model available from the EPR/ENDOR experiments and apparently the best model Wetmore et al.<sup>1</sup> have. Probably the inclusion of the hydrogen bonding scheme from the crystal structure<sup>3</sup> will alter the authors' calculations. In particular, the amino group will adopt a coplanar configuration which probably will increase the spin density at the C5 and N4 positions.

Wetmore et al.<sup>1</sup> next discuss a reduction product Sagstuen et al.<sup>2</sup> assigned as the N3–H protonated anion. Except for a 55 MHz hyperfine coupling from one of the –N4–H<sub>2</sub> protons, the theoretical spin densities closely agree with those computed from the experimental data. No proton confined to the pyrimidine ring plane (which it indisputably is in the single crystal) in a  $\pi$ -electron radical can give such a large hyperfine coupling. It must therefore be assumed that this proton was not confined to the molecular plane in the authors' energy minimization routine. If their conclusions are based on an out-of-plane proton, then they have little bearing on cytosine reduction products in single crystals and in DNA.

Finally, the authors examine radical structures from OH addition to C5=C6. They claim “...for the large couplings of the C5 and N1 protons in **C5OH** there is a fair match with the experimental sets assigned to **N1**”. Obvious problems with any suggestion that a C5 OH-adduct is a better model for the N1 deprotonated cation discussed above are (1) the directional properties of the hyperfine interactions from the ENDOR data clearly show that the major site of spin density for **N1** is C5 with the C5–H <sub>$\alpha$</sub>  bond in the molecular plane; (2) the EPR/ENDOR experiments showed no evidence for the 92.5 MHz  $\beta$ -proton coupling that **C5OH** would exhibit. Thus, the experimental data rules out identification of the **N1** radical as having the **C5OH** structure; as well, the low temperature conditions of the experiments virtually prohibit the OH-radical migration necessary for formation of **C5OH**.

In conclusion, the authors have impressive new calculations which generally agree nicely with experimental EPR/ENDOR results<sup>2</sup> from the primary reduction and oxidation products observed in irradiated cytosine derivatives. However, in a few cases, the authors' methods do not yield satisfactory hyperfine

coupling constants.<sup>4</sup> For this reason, it is important to recognize the limitations of these theoretical methods, and to emphasize that they should not be used uncritically to reject models well-substantiated by extensive and detailed experimental results.

**Acknowledgment.** This work is supported by PHS Grant R01 CA36810-12 awarded by the National Cancer Institute, DHHS. Partial support came from NATO Travel Grant 0426/88.

## References and Notes

- (1) Wetmore, S. J.; Himo, F.; Boyd, R. J.; Eriksson, L. A. *J. Phys. Chem.* **1998**, *B102*, 7464.
- (2) Sagstuen, E.; Hole, E. O.; Nelson, W. H.; Close, D. M. *J. Phys. Chem.* **1992**, *96*, 8269.
- (3) Jeffrey, G. A.; Kinoshita, Y. *Acta Crystallogr.* **1963**, *126*, 20.
- (4) The authors also present some data for the **N4** radical which are in disagreement with recent experimental results, see: Hole, E. O.; Nelson, W. H.; Sagstuen, E.; Close, D. M. *Radiat. Res.* **1998**, *149*, 109.