

# One-Electron Oxidation of 2'-Deoxyadenosine-5'-phosphate: Comparisons of Theoretical Calculations with Experimental Values

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A recent paper by Hou et al. (Hou, R.; Gu, J.; Xie, Y.; Yi, X.; Schaefer, H. F. *J. Phys. Chem. B* 2005, 109, 22053) on 2'-deoxyadenosine-5'-phosphate (5'-dAMP) reports calculations on one-electron oxidation of the 5'-dAMP anion. The paper presents a very interesting observation that, for the radical produced by electron removal, the unpaired spin density resides on both the phosphate and the adenine base moieties. There are also indications that this radical has a weakened C5'-O5' bond, and it is said that this may be the origin of a single-strand break in DNA. New calculations have been performed to show that the spin density on the phosphate is dependent on the charge on the phosphate. The use of the B3LYP method with the 6-31G(d) basis set yields results very similar to those obtained with the much larger B3LYP/DZP++ basis set in computing the structures of one electron oxidized 5'-dAMP. New calculations on the isotropic hyperfine couplings in 5'-dAMP are presented to show under just what conditions one might expect to see small amounts of unpaired spin density on the phosphates. Results show that this may occur in gas-phase studies of nucleotides but, most likely, not in DNA.

## Introduction

A recent paper by Hou et al. on 2'-deoxyadenosine-5'-phosphate (5'-dAMP) reports calculations on one-electron oxidation of the 5'-dAMP anion.<sup>1</sup> The paper presents a very interesting observation that, for the radical produced by one electron removal, the unpaired spin density resides on both the phosphate and the adenine base moieties. There are also indications that this radical has a weakened C5'-O5' bond, and it is said that this may be the origin of a single-strand break in DNA.

There are several questions that come to mind in reading the paper by Hou et al.<sup>1</sup> The authors are using the 5'-dAMP anion as a model. This molecule has a single proton on the phosphate group, and therefore the molecule bears a net negative charge. One-electron oxidation therefore produces a neutral species. In DNA the negative charge on the phosphate is usually neutralized by a counterion and several water molecules. So one question that comes to mind is whether or not the spin density on the phosphate is dependent on the charge on the phosphate. Does the negative charge on the phosphate attract a positive hole?

Also, Hou et al. use a B3LYP/DZP++ basis set that is especially suited for anionic species.<sup>1</sup> One should be concerned, however, that these diffuse functionals might be responsible for observation of delocalized spin density on both the base and the phosphate. Although calculations by Sevilla et al. on a nucleoside with just a 6-31G(d) basis set have clearly shown spin density on just the adenine base, it remains to be shown if the same conditions hold for a nucleotide.<sup>2</sup>

The optimized structure of the 5'-dAMP anion has the C2'-endo-C3'-exo conformation with the base in the anti arrangement. A picture of the author's optimized structure shows the phosphate and the base above the plane of the sugar. It seems from their picture as if one of the >P-O<sup>-</sup> oxygens is near the >C8-H hydrogen. One wonders what influence such an

intramolecular H-bond might have on the delocalization of the unpaired spin density onto the phosphate.

Hou et al. claim the unpaired spin density resides mainly in the lone-pair orbitals of the phosphate oxygens.<sup>1</sup> One has to ask about the effects of small amounts of unpaired spin density on the phosphate and whether or not this is a likely cause of a single-strand break. It is therefore necessary to examine the literature on the radiation damage products observed in DNA to see if phosphate radicals have been observed, and if so, under what conditions.

In their discussion of the structure of the one electron oxidized 5'-dAMP anion, Hou et al.<sup>1</sup> comment on the length of the P-O5' bond. In the optimized 5'-dAMP anion this bond is 1.418 Å and increases to 1.445 Å after electron loss. The authors suggest this might be a precursor to a single-strand break.

The paper by Hou et al. involves one-electron oxidation of a nucleotide.<sup>1</sup> Much is known about the radiation of chemistry of nucleotides. In general, oxidative products are found on the bases and tend to be stabilized by deprotonation. This should be particularly true for 5'-dAMP since the adenine cation radical is strongly acidic (pK<sub>a</sub> < 1).<sup>3</sup> The strong driving force for deprotonation makes the reaction independent of environmental conditions. The important point to consider here is that the calculations performed by Hou et al.<sup>1</sup> mimic gas-phase conditions. It remains to be seen if these findings are relevant to DNA.

All of these issues are addressed in the present paper. New calculations on the isotropic hyperfine couplings in 5'-dAMP are presented to show under just what conditions one might expect to see small amounts of unpaired spin density on the phosphates. Results show that this may occur in gas-phase studies of nucleotides but, most likely, not in DNA.

**Background Material: Radiation Chemistry of Adenine (Hyperfine Couplings).** The results of detailed electron paramagnetic resonance/electron nuclear double-resonance (EPR/ENDOR) experiments on nucleic acids constituents have played a major role in understanding the primary radiation effects (radical cations and radical anions) produced by ionizing

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radiation and the deprotonation, protonation reactions necessary to stabilize these defects. For a review see ref 4.

Ionizing radiation produces nonspecific ionizations; it ionizes DNA components approximately in direct proportion to the number of electrons on a given atom. The sugar–phosphate backbone contains 52% of the electrons; the “average base” contains 48%. EPR experiments have shown, however, that the final damage to DNA is not a random distribution among these three components. Rather, the majority of the radicals are on the DNA bases. What is clear from low-temperature experiments is that electron and hole transfer occurs after the initial random deposition of energy. So the question becomes, which initial ionization events will recombine and which ones will lead to a stably trapped radical. The answer often depends on the molecular environment.

The adenine radical cation was observed in a single crystal of adenine hydrochloride hemihydrate.<sup>5</sup> In this crystal, the adenine is protonated at N1. After electron loss, the molecule deprotonates at N1, giving  $\text{Ade}(\text{N1}+\text{H}, \text{N1}-\text{H})^{\bullet+}$ . This produces a radical that is structurally equivalent to the cation of the neutral adenine molecule with spin density on C8 and N10 ( $\rho(\text{C8}) = 0.17$  and  $\rho(\text{N10}) = 0.25$ ). The experimental hyperfine couplings are  $\text{C8}-\text{H}_\alpha = -13.7$  MHz,  $\text{N10}-\text{H1}_\alpha = 19.7$  MHz, and  $\text{N10}-\text{H2}_\alpha = 20.5$  MHz.

In single crystals of adenosine,<sup>6</sup> and anhydrous deoxyadenosine,<sup>7</sup> the N10 deprotonated cation ( $\text{Ade}(\text{N10}-\text{H})^\bullet$ ) is observed at 10 K, which is characterized by  $\rho(\text{C8}) = 0.16$  and  $\rho(\text{N10}) = 0.42$ . The experimental isotropic hyperfine couplings are  $\text{N10}-\text{H}_\alpha = 33.9$  MHz and  $\text{C8}-\text{H}_\alpha = -12.4$  MHz.

To date, there have been no reported high-level EPR/ENDOR experiments on irradiated adenine nucleotides. There is, however, a detailed ENDOR study of a guanine nucleotide in a single crystal of disodium deoxyguanosine-5'-phosphate tetrahydrate.<sup>8</sup> In that study, the  $\text{Gua}(\text{N10}-\text{H})^\bullet$  deprotonated guanine cation was observed at 10 K with 17.5%  $\pi$ -spin density at C8 and 33% nitrogen  $\pi$ -spin density at N10. This radical therefore has similar properties to the  $\text{Ade}(\text{N10}-\text{H})^\bullet$  radical observed in adenosine.

The experimental results suggest that one-electron oxidation of a purine nucleotide results first in hole transfer to the base (to form a radical cation) followed by deprotonation to form a stably trapped neutral radical. As noted above, this should be particularly true for adenine since the adenine cation radical is strongly acidic ( $\text{p}K_a < 1$ ).<sup>3</sup>

Next, it is important to look at the characteristics of one-electron oxidation of the phosphate. EPR studies on irradiated phosphates indicate a likely product would be the  $\text{PO}_4^{2-}$  radical with the unpaired electron primarily on the oxygen atoms interacting with the spin  $1/2$  nucleus of the  $^{31}\text{P}$ .<sup>9</sup> This radical is characterized by a ca. 30 G (84 MHz) doublet. Slightly larger hyperfine couplings of ca. 34 G (95 MHz) are expected for  $\text{HOPO}_3^-$  radicals, and 39 G (109 MHz) for  $(\text{HO})_2\text{PO}_2$  radicals, as the hole is forced onto a singly bonded oxygen.

Forward reactions of the one electron oxidized sugar–phosphate backbone are of two types: hole transfer to the base stack or formation of a neutral sugar radical by irreversible deprotonation. In the literature there is a bias toward the former process, since at one time there were no reports of sugar radicals in irradiated DNA. However, recent results have left little doubt that a substantial fraction ( $\sim 25\%$ ) of the holes are irreversibly trapped on the sugar.<sup>10</sup>

## Computational Methods

In the present study optimized geometries for the 5'-dAMP anion and its one electron oxidized radical were determined

using the B3LYP method. Calculations were carried out using the double- $\xi$  quality basis set with polarization and diffuse functions (denoted as DZP++) as described by Lee and Schaefer.<sup>11</sup> Tests were performed to see if similar results could be obtained using standard B3LYP/6-31G(d) calculations. All calculations were performed on the Gaussian 98 suite of programs.<sup>12</sup> Structures of molecules and spin densities are presented with ChemCraft.<sup>13</sup>

## Results and Discussion

**Use of the B3LYP/DZP++ Basis Set.** There is much discussion in the literature about the electron affinities (EAs) of the DNA bases. The measurement of the EAs has proved very challenging. Theoretical investigations at various levels of sophistication have produced a variety of results. Second-order Møller–Plesset perturbation theory with a modest basis set including diffuse functions yields negative adiabatic EAs for all the DNA bases.<sup>2</sup> Density functional theory (DFT) with large basis sets predict positive adiabatic EAs for uracil and thymine.<sup>14</sup>

More recently, a calibrated B3LYP/DZP++ basis set has been used to calculate the EAs of the 2'-deoxyribonucleosides. Calculated adiabatic EAs of dG, dA, dC, and dT are all positive in the presence of the 2'-deoxyribose.<sup>15</sup> Other calculations by Schaefer's group have applied the B3LYP/DZP++ basis to calculations on nucleotides.<sup>16</sup> Again this basis set seems to be a key to producing positive EAs for the nucleosides/tides.

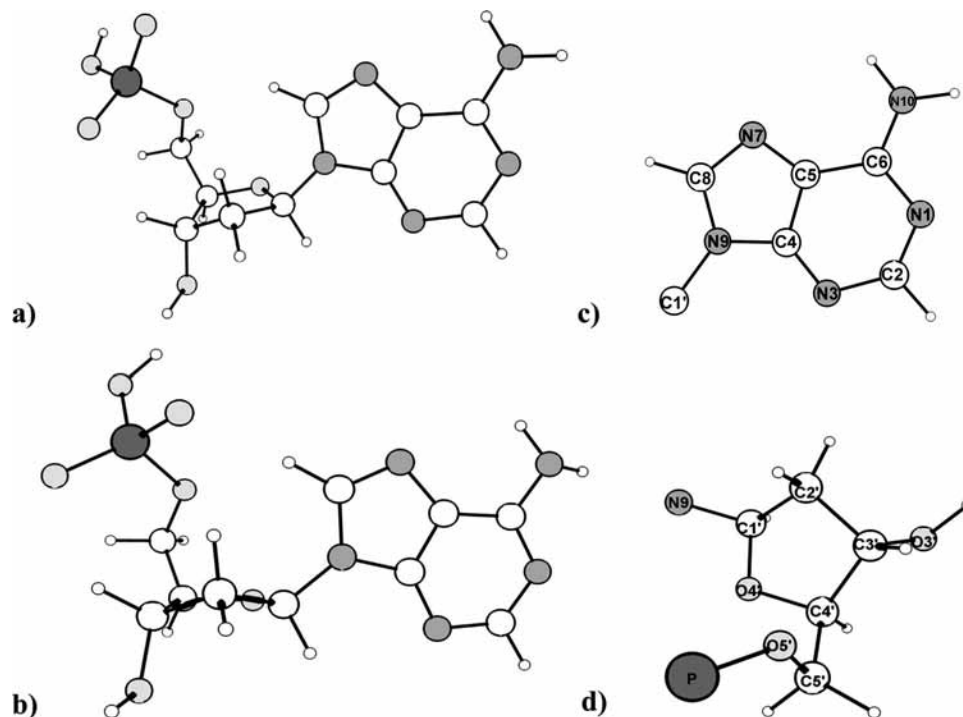
However, early on, Richardson et al. showed some obvious problems with using this B3LYP/DZP++ basis set.<sup>17</sup> For a calculation with just the adenine anion, the singly occupied molecular orbital (SOMO) showed a mixed character of a dipole and valence-bound anion. This work has been extended by Li et al.<sup>18</sup> They have looked at this problem in all of the DNA bases. Basically, they see that inclusion of diffuse functions in the basis set can result in contamination of the valence-bound state with the dipole-bound state. This was shown to be especially true for the purines.

To begin, it was necessary to locate the optimized geometry of the 5'-dAMP anion after one-electron oxidation reported by Hou et al.<sup>1</sup> Figure 1a shows the nonradical structure optimized with the DZP++ basis set. This molecule bears a negative charge on the phosphate. Hou et al.<sup>1</sup> call this the 5'-dAMP anion. One notes that the C8–H hydrogen is pointing at the O5' in the phosphoester bond in the optimization of the native molecule.

Figure 1b shows the optimized 5'-dAMP anion after one-electron oxidation, computed with the DZP++ basis set. This structure is therefore a neutral free radical. There are some changes in the molecular geometry upon oxidation. One sees the adenine  $\text{NH}_2$  has gone nonplanar, and now the C8–H is pointing toward the P–O1 oxygen.<sup>19</sup>

Since calculations with the DZP++ basis set are rather time-consuming, an optimization on the 5'-dAMP anion after one-electron oxidation was performed at the B3LYP/6-31G(d) level. The structure of the radicals optimized with the DZP++ and the 6-31G(d) basis sets are nearly identical. Although the DZP++ basis set seems well-suited for computing EAs, it seems that one does not need to perform time-consuming DZP++ calculations to study the structure of the oxidation product in 5'-dAMP.

In the Introduction, the importance of knowing hyperfine couplings to provide a firm link to actual experimental data was stressed. The theoretical work of Hou et al. reports that, for the radical produced by electron removal in 5'-dAMP, the unpaired



**Figure 1.** (a) 5'-dAMP anion optimized with the DZP++ basis set. (b) One electron oxidized 5'-dAMP anion optimized with the DZP++ basis set. (c) Atomic number scheme used here for the purine base. (d) Numbering scheme used here for the deoxyribo-phosphate.

**TABLE 1: Hyperfine Couplings for the 5'-dAMP Anion after One-Electron Oxidation**

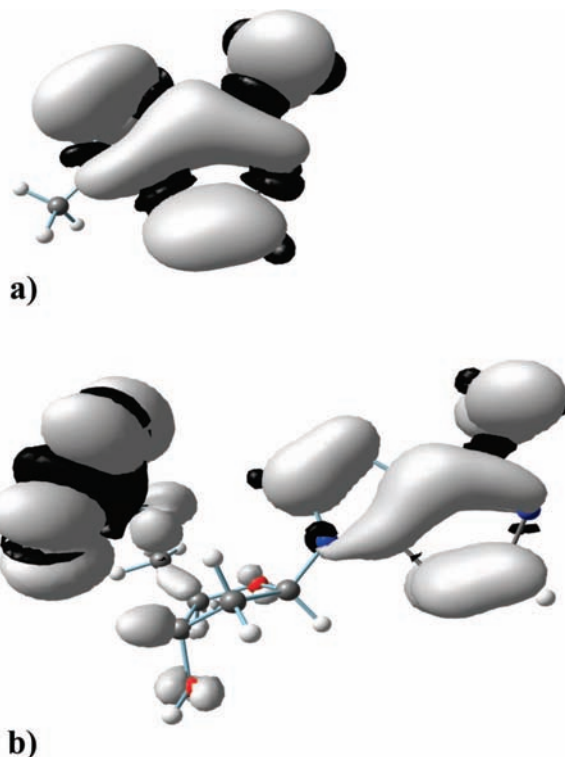
atom	B3LYP/DZP++ (MHz)	B3LYP/6-31G(d) (MHz)	exptl (MHz) <sup>a</sup>
N1	-0.59	-0.29	
C2-H	-2.18	-1.89	
N3	0.62	1.11	
N10	3.20	3.82	
N10-H1	-5.57	-5.41	19.7
N10-H2	-5.65	-5.46	20.5
C8-H	-4.11	-3.74	-13.7
C3'-H	6.06	8.95	
C4'-H	2.07	2.65	
<sup>31</sup> P	-103.96	-95.20	not detected

<sup>a</sup>These are actual experimental hyperfine couplings from the Ade(N1+H, N1-H)<sup>•+</sup> radical (ref 5).

spin density resides on both the phosphate and the adenine base moieties.<sup>1</sup> Since no hyperfine couplings are reported, it is difficult to relate this observation to available experimental data. The first step is to compute the hyperfine couplings and to examine the spin densities for the oxidation products in 5'-dAMP.

The computed isotropic hyperfine couplings for the anion after one-electron oxidation with the DZP++ and the 6-31G(d) basis sets are given in Table 1. One sees the results are nearly identical. For the current work therefore, most of the hyperfine couplings have been computed with the 6-31G(d) basis.

In the last column of Table 1 are the measured hyperfine couplings from one electron oxidized adenine. It is also evident in Table 1 is that the computed hyperfine couplings bear little resemblance to these experimental values. This is because the experimental values were determined from an actual adenine cation, whereas Hou et al.<sup>1</sup> claim that the radical produced by one-electron removal from the 5'-dAMP anion, the unpaired spin density resides on both the phosphate and the adenine base moieties.



**Figure 2.** (a) Spin density for the cation observed in 1-MeAdenine. (b) Spin density for the model presented by Hou et al. (refs 1 and 19).

First it is necessary to discuss the distribution of spin density in the calculations reported by Hou et al. as depicted in Figure 2. In both structures care has been taken to orient the base plane and the N9-C1' bond in the direction as shown in the ball and stick structure in Figure 1a. Also, the spins densities have both been drawn with the same isodensity contours.

Figure 2a shows the spin density for the cation observed in 1-MeAdenine. This is basically the model of the adenine cation

**TABLE 2: 5'-dAMP Cation (Charge-Neutralized Phosphate)**

atom	B3LYP/DZP++ (MHz)	B3LYP/6-31G(d) (MHz)	B3LYP/6-31G(d) Na + 3H <sub>2</sub> O (MHz)	exptl (MHz) <sup>a</sup>
N1	-0.89	-0.55 (-0.75) <sup>b</sup>	-0.82 (-0.70) <sup>c</sup>	
N3	4.68	7.38 (4.20)	6.75 (7.11)	
N7	2.28	4.54 (2.06)	5.33 (5.05)	
N9	-2.66	-1.86 (-2.29)	-1.75 (1.58)	
N10	10.15	13.44 (8.90)	12.80 (13.03)	
<sup>31</sup> P	-0.58	-0.44 (-0.46)	-0.33 (-0.17)	
C2-H	-8.63	-8.60 (-8.10)	-9.49 (-9.22)	
C8-H	-16.16	-16.76 (-15.46)	-16.52 (-16.40)	-13.7
N10-H1	-18.25	-19.58 (-17.31)	-18.70 (-19.02)	-19.7
N10-H2	-18.57	-19.86 (-17.66)	-18.96 (-19.28)	-20.5

<sup>a</sup> These are the experimental hyperfine couplings from the Ade(N1+H, N1-H)<sup>+</sup> radical (ref 5). <sup>b</sup> The numbers in parentheses were calculated at the B3LYP/6-311G(2df,p) level of theory. <sup>c</sup> The numbers in parentheses were calculated for the nucleotide in the B-DNA conformation.

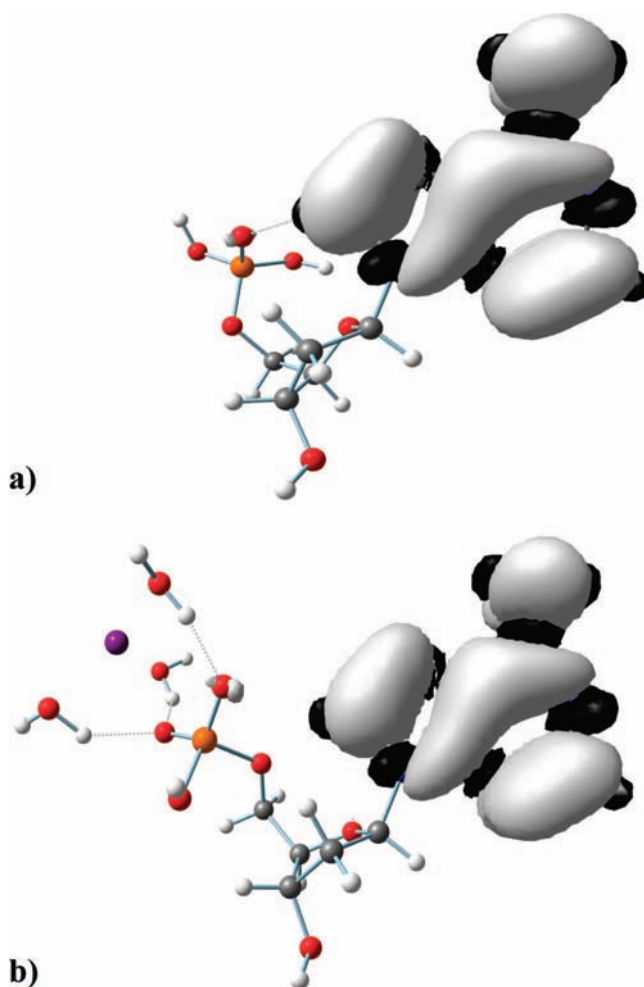
presented in the Introduction with major sites of spin density at C8 and N10. The model presented by Hou et al.<sup>1</sup> is shown in Figure 2b. Although the spin density on the adenine in the nucleotide appears to be similar to that shown for 1-MeAdenine, there are significant differences. Both parts a and b of Figure 2 were drawn with the same contour value. A closer inspection of Figure 2b shows that the spin density on the adenine is reduced. This is the reason why the computed C8-H<sub>α</sub> = -4.11 MHz, as compared to the C8-H<sub>α</sub> = -13.7 MHz for this same coupling determined experimentally for the adenine cation. This analysis therefore shows the importance of using the hyperfine couplings to have an understanding of spin densities.

There is one more point to mention about the oxidation product shown in Figure 2b. In their discussion of the structure of the oxidation product in the 5'-dAMP anion, Hou et al.<sup>1</sup> note that the electron is removed primarily from the HOMO-1-like orbital rather than from the HOMO (highest occupied molecular orbital). This is basically a problem with DFT calculations as one can see by comparing Hartree-Fock (HF) and Kohn-Sham orbitals. One notes that, in a calculation involving oxygen orbitals, the HF method has the p<sub>z</sub>-type orbitals higher in energy than the p<sub>x</sub>p<sub>y</sub>-type orbitals, whereas the opposite is true in a DFT calculation. Basically p<sub>z</sub>-type orbitals and p<sub>x</sub>p<sub>y</sub>-type orbitals are obtained by HF and DFT calculations, but with different energy orders.<sup>20</sup>

**Phosphate Hyperfine Couplings.** Table 1 shows that calculations on the one-electron oxidation product in the 5'-dAMP anion results in an appreciable <sup>31</sup>P hyperfine coupling (ca. 100 MHz). A coupling of this magnitude is very easy to detect by EPR. As discussed in the Introduction, this hyperfine coupling results from a PO<sub>4</sub><sup>2-</sup>-type radical with the unpaired electron primarily on the oxygen atoms interacting with the spin 1/2 nucleus of the <sup>31</sup>P. Although these radicals are easy to detect in irradiated model compounds of phosphates, they have never been detected in DNA or, indeed, in nucleotides.

In summary, the results presented in Table 1 are not in agreement with any known experimental data. The only experimental results available for one-electron oxidation of a purine have all the spin density on the base. There are no known experimental results in the nucleotides of any spin delocalized on the phosphate. It is therefore necessary to ask if such radicals can be stabilized in model compounds or in DNA.

In DNA the negative charge on the phosphate is usually neutralized by a counterion and several water molecules. So it is important to ask if the isotropic phosphate hyperfine coupling is dependent on the charge on the phosphate. As a first attempt, the negative charge on the phosphate can be neutralized by a P-OH proton. For the 5'-dAMP anion this produces a neutral



**Figure 3.** Effects of phosphate charge neutralizations. (a) The negative phosphate in 5'-dAMP neutralized with a proton. (b) The negative phosphate in 5'-dAMP neutralized by Na<sup>+</sup> + 3H<sub>2</sub>O.

molecule, and one-electron oxidation results in a cation. Results of these calculations are given in Table 2 (columns 2 and 3).

The first thing to observe in Table 2 is the effect of phosphate charge neutralization. In both columns 2 and 3 the <sup>31</sup>P hyperfine coupling is negligible. Figure 3a shows the spin density distribution in 5'-dAMP for the B3LYP/DZP++ calculation. One notes that all of the spin density has shifted to the adenine base. Indeed the spin distribution shown here is more like what one would expect of the adenine cation.<sup>21</sup>

One needs to stress here that the calculation of accurate hyperfine couplings normally requires larger basis sets, such as

**TABLE 3: 5'-dAMP with and without a  $-P-O\cdots H-C8$  H-Bond**

atom	H-bond (2.05 Å) Hfcc's (MHz)	no H-bond (3.77 Å) Hfcc's (MHz)
N1	3.40	1.99
N3	-0.22	0.96
N7	1.08	0.36
N9	-0.63	-0.18
N10	3.40	1.99
<sup>31</sup> P	-90.42	-106.1

6-311G(2df,p).<sup>22</sup> In Table 2, the third column shows the hyperfine coupling computed at the 6-31G(d) level and, in parentheses, the same calculations at the 6-311G(2df,p) level. Since there is hardly any difference between these two sets of calculations, for the present study hyperfine couplings were computed at the 6-31G(d) level of theory.

In the fourth column of Table 2 is a calculation with the phosphate anion neutralized with a sodium atom and three waters of hydration. This model was taken from the coordinates of the crystal structure of sodium guanylyl-3',5'-cytidine nonahydrate.<sup>23</sup> Variants of this model can be found in refs 24 and 25. This structure is shown in Figure 3b. Again one sees in Table 2 that neutralization of the phosphate charge results in a negligible <sup>31</sup>P hyperfine coupling.

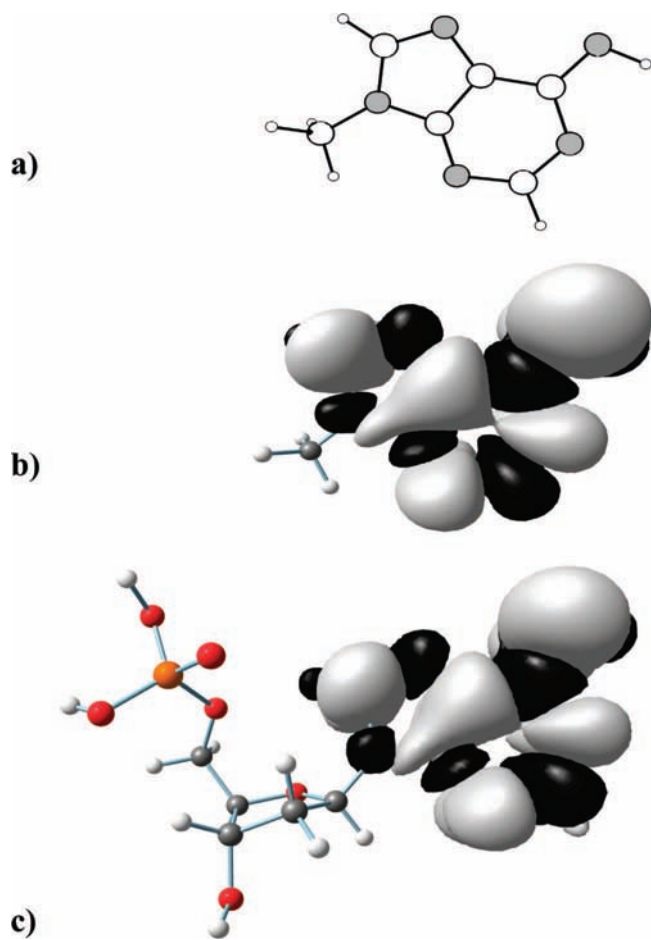
The optimized structure of the 5'-dAMP anion studied by Hou et al. has the C2'-endo-C3'-exo conformation with the base in the anti arrangement.<sup>1</sup> It is important to consider if the arrangement of the base and ribose makes a difference in the ability of the spin density to delocalize from the base to the phosphate. Therefore, a calculation was performed on deoxyadenosine monohydrate with the deoxyribose with C2'-endo-C3'-endo puckering taken from a crystal structure.<sup>26</sup> The basic difference here is with the O4'-C1'-N1-C8 torsion angle which is 77.4° for 5'-dAMP and -67.98° in deoxyribose. The computed hyperfine couplings for the deoxyribose structure are nearly identical to those presented in Table 2 for the 5'-dAMP one electron oxidized structure.

**Intramolecular H-Bonds.** The size and flexibility of 5'-dAMP suggest that multiple stable configurations may exist for the 5'-dAMP anion, for the neutral molecule, and for the one electron oxidized species. It is not the purpose of the present study to explore these multiple structures. One can see studies by Shishkin et al. for a discussion of the intramolecular H-bonds in various nucleotides.<sup>27</sup> It is important here to answer the question as to whether or not intramolecular H-bonds could promote the migration of spin density from the phosphate on to the base.

In the work of Hou et al. there does seem to be an H-bond between C8-H and one of the phosphate oxygens.<sup>1</sup> There is a concern that such a bond may facilitate transfer of spin density from the phosphate to the base. So first of all, the  $-P-O\cdots H-C8$  bond is 2.26 Å, which is too long to be considered an H-bond. In the one electron oxidized radical (Figure 1b), this distance is now 3.19 Å (actually the >C8-H is closest to the oxygen in the phosphoester bond (2.47 Å)). However, in both cases there are no contacts that could be considered H-bonds.

In looking at other geometries, several actual H-bonds have been observed. Table 3 shows a comparison of hyperfine couplings in a case of an H-bond between C8-H and one of the phosphate oxygen's and a case with no H-bond.

One sees only small changes in the computed hyperfine couplings. There is appreciable <sup>31</sup>P hyperfine coupling in both cases, so in this case at least, the delocalization of the spin



**Figure 4.** (a) Structure of the 9-MeAdenine amino deprotonated cation. (b) The spin density of 9-MeAdenine amino deprotonated cation. (c) Spin density of the 5'-dAMP amino deprotonated cation. (Note, here the phosphate has been charge-neutralized with a proton.)

density from the base to the phosphate is not facilitated by a hydrogen bond.

This study has been confined to geometries with the base in the anti conformation. As such, the base is near to the phosphate. In some of the optimization with the charge-neutralized phosphates, there were interesting intramolecular proton transfers. In these cases the deoxyribo-phosphate torsion angle was constrained to prevent the phosphate from approaching the adenine base.

**DNA Model Studies.** Calculations show that if the phosphate in 5'-dAMP bears a net negative charge, there is a tendency for the one-electron loss product to have spin density delocalized on both the phosphate and the adenine base. This gives rise to a ca. 100 MHz <sup>31</sup>P hyperfine coupling that has never been observed in DNA model studies. It is therefore likely that this species does not exist in either model compound studies or in one electron oxidized DNA.

As discussed in the Introduction, experimental results suggest that one-electron oxidation of a purine nucleotide results first in hole transfer to the base (to form a radical cation) followed by deprotonation to form a stably trapped neutral radical. Therefore calculations have been performed on the 5'-dAMP deprotonated cation.

Figure 4 shows the spin densities for the 5'-dAMP deprotonated cation. Figure 4b depicts the spin density of the deprotonated cation. Figure 4c shows the same product in 5'-dAMP.

The hyperfine couplings from each of these calculations are shown in Table 4. As one can see, the results are nearly

**TABLE 4: 5'-dAMP with Adenine NH<sub>2</sub> Deprotonated Cation**

atom	B3LYP/6-31G(d)		exptl (MHz)
	B3LYP/6-31G(d) (MHz) 5'-dAMP	(MHz) 5'-dAMP + Na <sub>3</sub> H <sub>2</sub> O	
N1	6.67	7.28	
C2-H	5.54	5.71	
N3	9.54	11.80	
N10	28.93	25.10	
N10-H1	-44.68 (36.69)	-40.45	-33.9
N7	-1.82	-2.19	
C8-H	-10.84 (-12.35)	-10.67	-12.4
N9	-1.64	-1.26	
<sup>31</sup> P	-0.02	-0.006	not detected

identical for the second column (5'-dAMP with a charge-neutralized phosphate and adenine N10-H deprotonated cation, for the third column (5'-dAMP + Na<sub>3</sub>H<sub>2</sub>O and the adenine N10-H deprotonated cation). As noted above, one-electron oxidation, followed by N10-H deprotonation, results in a neutral radical. Even with the small basis set used, the major hyperfine agree reasonably well with the experimental results. The agreement is even better when the hyperfine couplings are evaluated on the optimized cation using the 6-311G(2df,p) basis set (major couplings shown in parentheses in column 2 of Table 4).

**Strand Breaks.** As discussed in the Introduction, Hou et al. see an increase in the C5'-O5' bond length after one-electron oxidation of 5'-dAMP anion. However, the calculated change in bond lengths (from 1.418 to 1.445 Å) is small and bracket the average C5'-O5' bond length of 1.43 Å reported in summaries of nucleic acid structures in the Cambridge Structural Database.<sup>28</sup> The authors do not comment on the calculated P-O5' bond length of 1.677 Å, which is longer than the average length of 1.61 Å for this bond in the crystallographic data.

The new calculations presented here on the charge-neutralized 5'-dAMP nucleotide show only phosphoester bond lengths that are within the ranges reported in the Cambridge Structural Database. For example, after electron removal, the P-O5' bond length is 1.63 Å, and the O5'-C5' bond length is 1.43 Å. There are thus no indications of any phosphoester bond lengthening in the charge-neutralized 5'-dAMP nucleotide.

## Conclusions

A number of questions were posed in the Introduction about calculations performed on the one-electron oxidation product of the 5'-dAMP anion. The answers to these questions have been presented here and can be summarized as follows.

The location of spin density on the phosphate moiety of the 5'-dAMP nucleotide is highly dependent on the charge state of the phosphate. One-electron oxidation of the 5'-dAMP anion produces the situation shown in Figure 2b with spin density shared between the phosphate moiety and the adenine base. However, if the charge on the phosphate is neutralized with an additional P-OH proton, or with a counterion, the spin density shifts to the adenine base to give the familiar adenine cation.

The paper by Hou et al. uses the B3LYP method with the DZP++ basis set to calculate the properties of the one-electron oxidation of the 5'-dAMP nucleotide.<sup>1</sup> It has been shown here that one can obtain nearly the same results with the B3LYP method using the 6-31G(d) basis set. Essentially the same hyperfine couplings are calculated with either method for one-electron oxidation of the 5'-dAMP anion or for the charge-neutralized 5'-dAMP nucleotide.

For a flexible molecule like the 5'-dAMP nucleotide it is important to know if stable configurations involving intramolecular H-bonds influence the migration of spin density to the phosphate moiety. For the geometries considered in this work, it appears that intramolecular H-bonds are not important. It appears that spin density on the phosphate is determined by the charge on the phosphate. Simply put, a negative charge on the phosphate attracts a positive hole.

A major feature of the present work has been the discussion of use of hyperfine couplings in determining free radical structures. It has been shown how useful it is to know the hyperfine couplings of the various radicals discussed here in order to understand where spin densities are located and how these results can be compared with experimental data. For example, it is well-known that the one-electron oxidation of a phosphate results in an appreciable <sup>31</sup>P hyperfine coupling (ca. 100 MHz). Although these radicals are easy to detect in irradiated model compounds of phosphates, they have never been detected in DNA or, indeed, in nucleotides.

A final point has been the discussion of the use of model compounds in an effort to understand the radiation chemistry of DNA. Results show that one expects to see some unpaired spin density on the phosphate as the results of one-electron oxidation of the 5'-dAMP anion. These results are therefore relevant to the gas-phase studies of nucleotides but, most likely, not to DNA.

In DNA the negative charge on the phosphate is neutralized by a counterion. Results presented here show that one-electron oxidation of the neutral 5'-dAMP nucleotide results in a product with all of the spin density on the adenine base (forming the adenine cation). In model compounds the adenine cation is stabilized by deprotonation at the exocyclic NH<sub>2</sub> even at helium temperatures.<sup>6</sup> In DNA the initial hole produced randomly by one-electron oxidation is mobile and will move about until it encounters a deep hole trap on a purine base. This then points to an important scheme nature uses to protect DNA from oxidative damage. Although one-electron oxidation of phosphate is likely to occur, no damage occurs to the phosphoester bond once the hole is sequestered on a purine base.

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