Significant Effects of Phosphorylation on Relative Stabilities of DNA and RNA Sugar Radicals: Remarkably High Susceptibility of H-2′ Abstraction in RNA

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The roles of nucleic acid radicals in DNA and RNA damage cannot be properly understood in the absence of knowledge of the C−H bond strengths depicting the energy cost to generate each of these radicals. However, previous theoretical studies on the relative energies of different nucleic acid radicals are not fully convincing mainly because of the use of oversimplified model compounds. In the present study we chose nucleoside 3′,5′-bisphosphates as model compounds for DNA and RNA, in which the effects of both the nucleobase and phosphorylation were taken into consideration. Using the newly developed ONIOM-G3B3 methods, we calculated the gas-phase bond dissociation enthalpies and solution-phase bond dissociation free energies of all the carbohydrate C−H bonds in the model compounds. It was found that the monoanionic phosphate group (OPO_3H^-) was a better radical stabilization group than the OH group by 1.3 kcal/mol, whereas the neutral phosphate group (OPO_3H_2) was a significantly worse radical stabilization group than OH by 4.4 kcal/mol. Due to these reasons, the relative thermodynamic susceptibility of H-abstraction from deoxyribonucleotides and ribonucleotides varied considerably depending on the phosphorylation state and the charge carried by the phosphate groups. Strikingly, the bond dissociation free energy of C2′−H in ribonucleotides was dramatically lower than that of all the other C−H bonds by 5−6 kcal/mol regardless of the phosphorylation state and the charge carried by the phosphate group. This explained the previous experimental finding that radiation damage of RNA occurs mainly via H-abstraction at H-2′. A model study suggested that the strength of the hydrogen bonding interaction between the 2′-OH and 3-phosphate groups should dramatically increase from ribonucleoside 3′,5′-bisphosphate to its C2′ radical. The strengthened hydrogen bonding stabilized the C2′ radical, rendering the C2′−H bond of RNA extraordinarily vulnerable to H-abstraction.

1. Introduction

Intracellular free-radical oxidants including certain antibiotics, metal complexes, oxygen metabolites, and molecules ionized by high-energy radiation are well-known instigators of DNA and RNA damage.[1] The damage can take place at the heterocyclic bases of nucleic acids, producing a variety of nucleobase radicals that may rearrange to yield an abasic site. Concurrently, abstraction of a hydrogen atom from ribose or deoxyribose by the oxidants produces a carbon-based sugar radical that can rearrange, culminating in scission of the nucleic acid strand. The radical-mediated nucleic acid lesions may contribute to a variety of health-related problems including mutagenesis, carcinogenesis, aging, and inherited disease.[1] During the past decade the research on DNA and RNA radicals has literally exploded. A focus of the research is to understand the mechanistic pathways by which oxidative agents cleave DNA and RNA.[2]

A deoxyribose residue in the backbone of DNA has seven H-atoms attached to carbon which, in principle, are available for abstraction by an oxidizing agent. Ribose in RNA has six such H-atoms, since one hydrogen atom at the 2′-carbon has been replaced by a hydroxyl group. A key question in elucidating the mechanism of nucleic acid degradation through the sugar radical pathway is which of these H-atoms is preferentially abstracted in the formation of a strand break.[1,2] Previous studies have established that the thermodynamic susceptibility of H-abstraction have a strong correlation with the C−H bond strength.[3−5] This raises an important question as to the bond dissociation enthalpies (BDEs) of the various C−H bonds in nucleic acids. Because experimental determination of C−H BDEs of nucleic acids has been a formidable challenge that will not be resolved soon, during the past several years a number of theoretical methods have been utilized to predict the C−H BDEs of nucleic acid models at different levels of sophistication.[3−7]

In 1994 Osman et al. calculated the energy of H-abstraction for positions 1′−4′ of 2-deoxy-D-ribose using the MP2/6-31G* method.[3] It was found that abstraction of H-1′, H-3′, or H-4′ required similar amounts of energy, whereas abstraction of H-2′ required more energy. One year later Colson and Sevilla calculated model sugars such as deoxyribose diphosphate at the ROHF/3-21G level.[4] They showed that abstraction of H-1′ was energetically favorable in these molecules, while abstraction of H-2′ was least favorable. In 1999 Osman et al. utilized the HF/6-31G method to study ribose and its radicals produced by H-abstraction from ring carbons.[5] It was found that in distinction from deoxyribose, the BDEs of the various hydrogens of ribose are all of comparable strength. Finally, in 2002 Melikyan et al. calculated the relative stabilities of thymidine-3′,5′-diphosphoric acid derived isomeric radicals using the B3LYP/6-31G*//HF/3-21G* method.[6] The most probable sites of an H-atom (4′, 5′) abstraction were identified.[6] This finding was in contrast with the earlier results reported by Colson and Sevilla.[4]

All the above studies have made great contributions to the understanding of radical-mediated DNA and RNA lesions. Nonetheless, at the present time one still cannot be confident

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about the relative thermodynamic susceptibility of H-abstraction at each position of the nucleic acids. The reasons are 3-fold: (1) in most previous studies only the sugar moieties of nucleic acids were modeled, while the effects of nucleobases and phosphate groups on the radical stability were not considered; (2) fairly low levels of theoretical methods were utilized in the previous studies, and some of these methods (e.g., HF/6-31G) have been shown to be unreliable for handling organic radicals;[8] and (3) the results from different previous studies are not fully consistent with each other. Melikyan et al. showed that the most probable sites of H-abstraction in thymidine-3′,5′-diphosphoric acid were H-4′ and H-5′,[6] but Colson and Sevilla showed that abstraction of H-1′ was the most favorable in deoxyribose diphosphate.[4]

It is obvious that more compelling studies are needed about the relative thermodynamic susceptibility of H-abstraction at each position of nucleic acids. However, before such studies can be conducted, a major obstacle is the lack of theoretical tools that can handle systems as large as nucleic acid radicals within the level of "*chemical accuracy*" (ca. 1−2 kcal/mol). Very recently we developed an ONIOM-G3B3 method that can deal with organic molecules and radicals containing 20−50 non-hydrogen atoms.[9] By comparing with over 60 experimental BDEs of diverse sizable molecules it was shown that the accuracy of ONIOM-G3B3 is around 1.4 kcal/mol. Using the ONIOM-G3B3 method we studied the radicals derived from ribonucleosides and deoxyribonucleosides that do not have the phosphate groups.[9] It was predicted that the probability of H-abstraction from deoxyribonucleosides in the gas phase decreases in the order H-1′ > H-5′ > H-4′ ∼ H-3′ > H-2′, whereas the probability of H-abstraction from ribonucleosides in the gas phase decreases in the order H-1′ ∼ H-2′ ∼ H-4′ ∼ H-5′ > H-3′.

It was our belief then that the ONIOM-G3B3 BDEs of nucleosides were sufficient to predict the relative thermodynamic susceptibility of H-abstraction in the radical-mediated DNA and RNA lesions. Nonetheless, because 3′,5′-diphosphorylated nucleotides are more realistic models for DNA and RNA than nucleosides, we recently re-performed ONIOM-G3B3 calculations on 3′,5′-diphosphorylated nucleotides and their radicals. In the beginning we expected to see no significant difference between the nucleoside and nucleotide radicals because the phosphate groups are separated from the radical center by at least one σ bond. However, after the calculation was completed, we were very surprised to find that the relative stabilities of 3′,5′-diphosphorylated nucleotide radicals are dramatically different from the relative stabilities of nucleoside radicals. This unexpected finding is significant not only because it reveals intriguing phosphorylation effects on the stabilities of organic radicals, but also because it implies the importance of choosing phosphorylated model compounds in the study of the problems associated with DNA and RNA radicals. Furthermore, we discovered, for the first time, that the C2′−H bond of the phosphorylated ribonucleotides is extraordinarily vulnerable to H-abstraction due to the hydrogen-bonding interaction between the 2′-OH and 3′-phosphate groups. This novel finding can be used to interpret some puzzling experimental observations reported in the past.

2. Computational Methodology

Ab initio calculations were performed with the Gaussian 03 suite of programs.[10] Conformation analyses and geometry optimizations were performed with the standard B3LYP/6-31G(d) method. Frequency calculations were carried out at the B3LYP/6-31G(d) level of theory and performed on all of the species to confirm convergence to appropriate local minima of the energy surface. Single-point electronic energies were then calculated at the ONIOM(MP4(FC)/6-31G(d):B3LYP/6-31G(d)), ONIOM(MP2(FC)/6-31G(d):B3LYP/6-31G(d)), ONIOM(MP4(FC)/6-31+G(d):B3LYP/6-31G(d)), ONIOM (MP2(FC)/6-31+G(d):B3LYP/6-31G(d)), ONIOM(MP4(FC)/6-31G(2df,p):B3LYP/6-31G(d)), ONIOM(MP2(FC)/6-31G(2df,p):B3LYP/6-31G(d)), ONIOM(QCISD(T,FC)/6-31G(d):B3LYP/6-31G(d)), and ONIOM(MP2(FU)/G3large:B3LYP/6-31G(d)) levels. The ONIOM-G3B3 energy was then obtained from the above serious of energies by using the equation discussed in our previous work.[9]

Next the ONIOM-G3B3 energies were converted to the enthalpy values at 298 K, 1 atm, by adding the standard temperature correction terms H_{trans}, H_{rot}, and H_{vib}. These terms are calculated at the B3LYP/6-31G(d) level by using the equilibrium statistical mechanics with harmonic oscillator and rigid rotor approximations. Finally, the bond dissociation enthalpy was calculated as the enthalpy change of the reaction A−B (g) → A$^{\bullet}$ (g) + B$^{\bullet}$ (g) in the gas phase at 298 K, 1 atm.[11]

Free energy of solvation was calculated by using the conductor-like polarizable continuum model (CPCM), as implemented in Gaussian 03.[12] PCM methods used here represent the solute as a cavity made up of a set of interlocking spheres. The cavity is built by the UAKS model. In this model a sphere is put around each solute heavy atom by using the UATM atomic radii. Hydrogen atoms are enclosed in the sphere of the atom to which they are bonded. All the CPCM calculations were performed at the B3LYP/6-31G(d) level. Both the electrostatic and nonelectrostatic contributions were included for the total solvation energies.

Finally, for the model hydrogen bonding complexes the structures were optimized by using the MP2/6-31+G(d) method. Single-point calculations were then performed at the MP2/6-311++G(3df,3pd) level. The interaction energies were corrected with zero point energy at the MP2/6-31+G(d) level. The interaction energies were also correct with the basis set superposition errors (BSSE) estimated by using the counterpoise technique.[13]

3. Results

3.1. Geometry Optimization. The carbohydrate C−H bonds of eight nucleotide molecules are investigated here. These molecules include deoxyadenosine 3′,5′-bisphosphate, deoxyguanosine 3′,5′-bisphosphate, deoxycytidine 3′,5′-bisphosphate, deoxythymidine 3′,5′-bisphosphate, adenosine 3′,5′-bisphosphate, guanosine 3′,5′-bisphosphate, cytidine 3′,5′-bisphosphate, and uridine 3′,5′-bisphosphate. Two patterns of ionization states are considered to model DNA and RNA in the aqueous buffers. In the first pattern, all the model compounds have no proton removed. Thus in the first pattern all the nucleotides are neutral compounds. In the following discussions the nucleotides in the first pattern (or, neutral pattern) are symbolized as dApp, dCpp, dGpp, dTpp, rApp, rCpp, rGpp, and rUpp. In the second pattern each phosphate group has one, and only one, proton removed. In consequence every model compound in the second pattern has two negative charges. In the following discussions the nucleotides in the second pattern (or, negative pattern) are symbolized as dApp^{2-}, dCpp^{2-}, dGpp^{2-}, dTpp^{2-}, rApp^{2-}, rCpp^{2-}, rGpp^{2-}, and rUpp^{2-}.

Since nucleic acids present a conformational challenge, some explanation as to the rationale for the initial geometries is warranted. First, because the B DNA form (i.e., the deoxyribose pseudorotational state = S-type, C2′-endo) is considered the most important one for biological systems,[15] we optimize

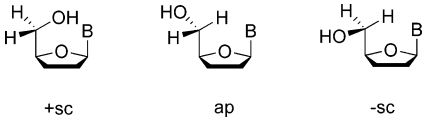


Figure 1. The +sc, ap, and −sc conformations with regard to the rotation of C4′−C5′.

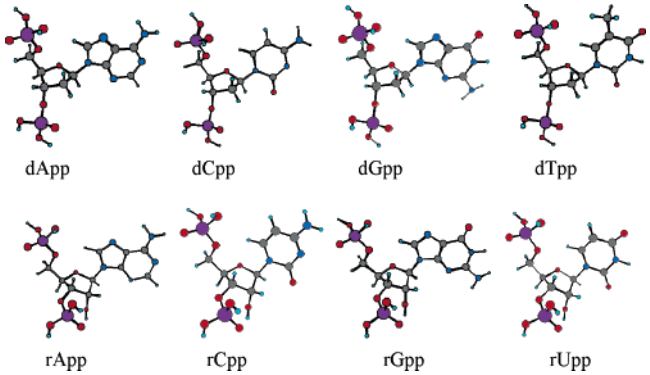


Figure 2. The fully optimized structures at the B3LYP/6-31G(d) level for dApp, dCpp, dGpp, dTpp, rApp, rCpp, rGpp, and rUpp.

deoxyribonucleoside 3′,5′-bisphosphates starting from a conformation identical with the B form of DNA. On the other hand, because the A form of RNA (i.e., the ribose pseudorotational state = N-type, C3′-endo) is considered the most important one for biological systems,[15] we optimize ribonucleoside 3′,5′-bisphosphates starting from a conformation identical with the A RNA form.

Second, with regard to the orientation of nucleobase there are two possible conformations as reflected by the χ torsion angel (i.e., O4′−C1′−N1−C2 for pyrimidine or O4′−C1′−N9−C4 for purine). When the C2 or C4 atom of the nucleobase is away from the sugar moiety the conformation is anti. Otherwise the conformation is syn. The anti conformation has the smaller H6 (pyrimidine) or H8 (purine) atom above the sugar ring, while the syn conformation has the larger O2 (pyrimidine) or N3 (purine) in that position. The previous studies have suggested that the anti conformation should be favored for nucleotides under most conditions.[14] Thus in the present study we consider the anti conformation only.

Third, due to the rotation of the C4′−C5′ bond we can generate three different conformations named +sc (synclinal), ap (antiperiplanar), and −sc for C5′-OH in each nucleoside 3′,5′-bisphosphate (see Figure 1). In the previous studies we found that all the nucleosides favor the +sc conformation by comparing the energies of the three different conformers. This finding is in agreement with the experimental studies on both nucleosides and nucleotides.[16] The driving force for the stabilization of the +sc conformation appears to be the C(from nucleobase)−H···O5′ hydrogen bond (Also see Figures 2 and 3).[17] Accordingly, in the present study we consider the +sc conformation only.

Finally, due to the rotations of the O3′−P and O5′−P bonds there are nine possible conformations for each nucleoside 3′,5′-bisphosphate (see Table 1). Utilizing the B3LYP/6-31G(d) method, we optimize each of the conformations and compare their energies. It is found that for dApp, dCpp, dGpp, dTpp, rApp, rCpp, rGpp, and rUpp in the neutral pattern, the (+60°, −100°) conformation is always favored. On the other hand, for the negative pattern $dApp^{2-}$, $dCpp^{2-}$, $dGpp^{2-}$, and $dUpp^{2-}$ favor the (+60°, −100°) conformation, $rApp^{2-}$ and $rGpp^{2-}$ favor the (180°, +20°) conformation, whereas $rCpp^{2-}$ and $rUpp^{2-}$ favor the (+60°, +20°) conformation. Utilizing the lowest energy

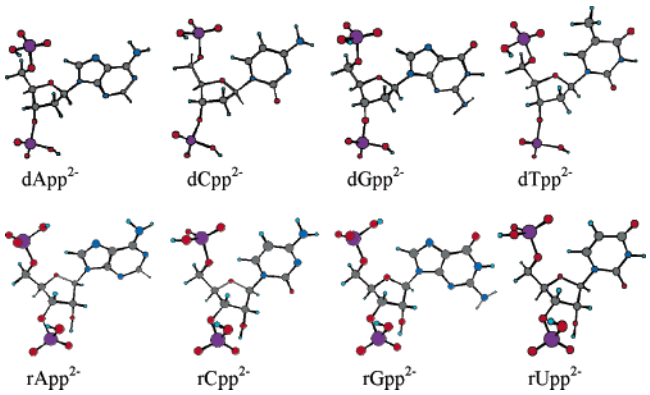


Figure 3. The fully optimized structures at the B3LYP/6-31G(d) level for $dApp^{2-}$, $dCpp^{2-}$, $dppG^{2-}$, $dTpp^{2-}$, $rApp^{2-}$, $rCpp^{2-}$, $rGpp^{2-}$, and $rUpp^{2-}$.

conformations, we then fully optimized dApp, dCpp, dGpp, dTpp, rApp, rCpp, rGpp, and rUpp (see Figure 2) and $dApp^{2-}$, $dCpp^{2-}$, $dGpp^{2-}$, $dTpp^{2-}$, $rApp^{2-}$, $rCpp^{2-}$, $rGpp^{2-}$, and $rUpp^{2-}$ (see Figure 3) using the B3LYP/6-31G(d) method. The detailed geometry parameters (bond lengths, bond angles, and dihedron angles) can be found in the Supporting Information.

Using similar approaches we next investigate the structures of all the sugar radicals of the nucleotides. For deoxyribonucleoside 3′,5′-bisphosphate radicals, we only consider the biorelevant C2′-endo/anti/+sc conformation but we allow the O3′−P and O5′−P bonds to freely rotate. For ribonucleoside 3′,5′-bisphosphate radicals, we only consider the biorelevant C3′-endo/anti/+sc conformation but allow the O3′−P and O5′−P bonds to freely rotate. By comparing the energies of all the possible conformations under the aforementioned restrictions at the B3LYP/6-31G(d) level, we are able to generate the most favorable conformation for each sugar radical. Once the conformation is determined, the remaining geometry parameters are then fully optimized by using the B3LYP/6-31G(d) methods. The detailed structural information for each sugar radical can be found in the Supporting Information.

3.2. Gas-Phase Bond Dissociation Enthalpies. The carbohydrate C−H BDEs of the nucleotide molecules are calculated by using the ONIOM-G3B3 methods. In the calculations each molecule is partitioned into two layers. The core layer, which contains the target C−H bond undergoing homolysis, is treated with the G3B3 method. The effect of the external layer on total energy is then calculated by using the B3LYP/6-31G(d) method. The integrated total energy of the nucleotide molecule or the corresponding radical is defined as

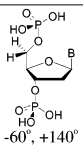
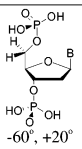
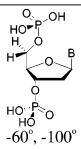
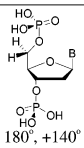
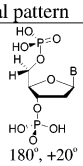
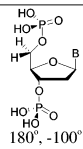
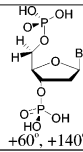
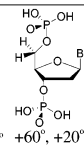
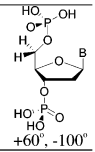
$$E(\text{ONIOM-G3B3}) = E(\text{G3B3, core}) + E(\text{B3LYP, external}) - E(\text{B3LYP, core})$$

The C−H BDE is then calculated as the energy difference between the nucleotide molecule and the radical, corrected by the standard temperature (298 K) correction terms (H_{trans}, H_{rot}, and H_{vib}) calculated at the B3LYP/6-31G(d) level. It is worth mentioning that the success of the ONIOM-G3B3 method in the BDE calculation can be attributed to the fact that the bond dissociation is a rather local phenomenon.[18] While very high levels of electron correlation are required for the local region of the bond to be broken, often the effects of the remote substituent atoms can be handled with a lower level of electron correlation treatment.

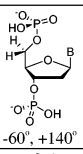
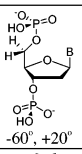
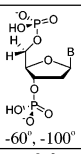
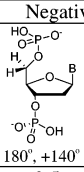
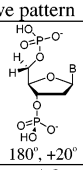
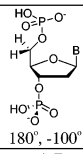
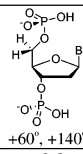
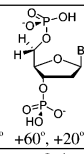
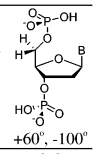
The detailed partitionings of layers for each C−H bond in the nucleoside 3′,5′-bisphosphates are shown in the Supporting Information. These partitionings obey the following rules: (1)

TABLE 1: Relative Energies of the Nine Possible Conformations with Regard to the Rotations of O−P Bonds for Each Nucleoside 3′,5′-Bisphosphate (kcal/mol)[a,b]

Neutral pattern

Species	 -60°, +140°	 -60°, +20°	 -60°, -100°	 180°, +140°	 180°, +20°	 180°, -100°	 +60°, +140°	 +60°, +20°	 +60°, -100°
dApp	+1.6	+0.3	nd	+2.4	+0.9	+1.0	0.0	+0.2	**0.0**
dCpp	nd	nd	+0.7	+1.4	nd	+0.8	+0.6	nd	**0.0**
dGpp	+2.1	+0.9	+0.6	+1.8	+1.4	+0.1	+1.5	+0.1	**0.0**
dTpp	+1.3	0.0	0.0	+2.2	+0.9	+0.9	+1.4	0.0	**0.0**
rApp	nd	+1.7	nd	+4.1	nd	+0.7	nd	nd	**0.0**
rCpp	nd	+2.3	+0.4	+3.5	nd	+0.2	nd	nd	**0.0**
rGpp	nd	nd	nd	+4.4	nd	+1.0	nd	nd	**0.0**
rUpp	nd	+2.3	+0.2	+4.2	nd	+0.6	nd	nd	**0.0**

Negative pattern

Species	 -60°, +140°	 -60°, +20°	 -60°, -100°	 180°, +140°	 180°, +20°	 180°, -100°	 +60°, +140°	 +60°, +20°	 +60°, -100°
$dApp^{2-}$	+3.1	+2.6	+2.3	+2.5	+1.8	+1.7	+0.8	+0.4	**0.0**
$dCpp^{2-}$	+4.0	+3.4	+2.9	+1.9	+1.6	+0.7	+1.1	+0.6	**0.0**
$dGpp^{2-}$	+1.9	+1.0	+1.3	+1.5	+0.7	+1.0	+0.8	+0.1	**0.0**
$dTpp^{2-}$	+2.9	+2.3	+2.1	+2.0	+1.5	+1.0	+0.8	+0.3	**0.0**
$rApp^{2-}$	+3.3	+1.1	+6.4	+1.9	**0.0**	+5.0	+2.6	+0.5	+5.6
$rCpp^{2-}$	+3.1	+1.2	+5.9	+2.8	+1.1	+5.7	+1.7	**0.0**	+4.4
$rGpp^{2-}$	+4.3	+1.9	+7.6	+2.1	**0.0**	+5.3	+2.9	+1.2	+4.8
$rUpp^{2-}$	+3.1	+1.2	+6.1	+2.4	+0.5	+5.3	+1.8	**0.0**	+4.6

[a] In some of the optimizations the $C_4'-C_5'-O_5'-P_5'$ dihedral angle differs from 174.41° by over 20°. Since this is not considered to be permissible in DNA and RNA (see: Colson, A.; Sevilla, M. D. *J. Phys. Chem.* **1995**, *99*, 3867), we did not calculate the corresponding energies (labeled as "nd" in the table). [b] In each row the energy of the most stable conformation is defined as zero. The energies for other conformations are then reported as relative values.

TABLE 2: The C−H BDEs (kcal/mol) for Nucleoside 3′,5′-Bisphosphates (in both neutral and negative patterns) and Nonphosphorylated Nucleosides (symbolized as dA, dC, dG, dT, rA, rC, rG, rU) in the Gas Phase As Calculated by the ONIOM-G3B3 Method

compd	$C_1'-H$	$C_2'-H$	$C_3'-H$	$C_4'-H$	$C_5'-H$
dApp	94.2	101.1	101.9	94.8	100.6
$dApp^{2-}$	95.4	102.4	100.1	97.7	94.5
dA	94.3	101.4	98.0	97.8	96.8
dCpp	95.0	100.7	101.3	94.2	100.9
$dCpp^{2-}$	97.1	100.9	100.0	97.2	94.8
dC	94.6	101.1	97.6	97.0	95.7
dGpp	94.6	101.2	102.0	94.7	100.4
$dGpp^{2-}$	95.3	102.3	100.2	97.4	94.2
dG	95.0	101.4	98.2	98.1	96.8
dTpp	96.3	100.7	101.4	95.1	100.5
$dTpp^{2-}$	96.5	101.9	99.9	98.2	94.9
dT	95.6	101.4	97.9	97.5	96.2
rApp	93.9	90.7	100.4	95.6	100.4
$rApp^{2-}$	95.4	88.8	96.3	97.5	93.9
rA	96.5	97.3	98.9	96.1	96.2
rCpp	94.4	91.5	100.7	95.5	100.6
$rCpp^{2-}$	97.7	89.1	95.9	97.3	94.9
rC	95.0	97.7	98.8	95.9	96.2
rGpp	95.6	91.2	100.5	95.7	100.3
$rGpp^{2-}$	93.0	89.2	96.8	97.5	93.6
rG	96.6	94.1	99.0	96.1	96.1
rUpp	97.3	90.2	100.6	96.1	100.5
$rUpp^{2-}$	96.9	88.7	96.1	97.9	94.5
rU	97.9	96.3	98.9	96.5	96.1

because G3B3 is a highly resource demanding method, the core layer contains six non-hydrogen atoms; (2) in the partitioning a double bond cannot be ruptured, thus either the two atoms of a double bond are both included in the core layer or they are both excluded; and (3) because α-substituents usually have more significant effects on BDEs than the *β*-substituents, we consider the α-substituents at first, and similarly, we consider the *β*-substituents before we consider the *γ*-substituents. Using the partitioning methods as described above, we calculate the C−H BDEs for the nucleoside 3′,5′-bisphosphate in the gas phase with the ONIOM-G3B3 method (see Table 2). The corresponding C−H BDEs previously calculated for nucleosides at the ONIOM-G3B3 level are also included in Table 2 for comparison. It can be seen that the introduction of the phosphate groups changes the strengths of most C−H bonds by 1−3 kcal/mol. More strikingly, the C2′−H BDEs of ribonucleosides are dramatically reduced by 5−8 kcal/mol due to the phosphorylation in either the neutral or negative pattern.

3.3. Solution-Phase Bond Dissociation Free Energies. Compared to the gas-phase BDEs, the solution-phase bond dissociation free energies are more relevant to the experimental realities. To calculate the solution-phase bond dissociation free energies, we consider the thermodynamic cycle as shown in Scheme 1. In the scheme, ΔG_{aq} is the solution-phase bond

SCHEME 1

$$\begin{array}{ccccc} \text{A-H}_{gas} & \xrightarrow{\Delta G_{gas}} & \text{A}\bullet_{gas} & + & \text{H}\bullet_{gas} \\ \downarrow \Delta G_s(\text{AH}) & & \downarrow \Delta G_s(\text{A}\bullet) & & \downarrow \Delta G_s(\text{H}\bullet) \\ \text{A-H}_{aq} & \xrightarrow{\Delta G_{aq}} & \text{A}\bullet_{aq} & + & \text{H}\bullet_{aq} \end{array}$$

dissociation free energy. This quantity can be calculated from ΔG_{gas} (gas-phase bond dissociation free energy) and ΔG_s(AH),

TABLE 3: The C−H Bond Dissociation Free Energies (kcal/mol) in Aqueous Solutions for Deoxyribonucleoside 3′,5′-Bisphosphates and Ribonucleoside 3′,5′-Bisphosphates

compd	C_1'−H	C_2'−H	C_3'−H	C_4'−H	C_5'−H
dApp	92.1	97.2	94.9	88.7	96.0
dCpp	91.2	96.9	94.5	89.8	95.9
dGpp	93.2	96.7	94.6	88.7	95.7
dTpp	91.3	96.3	94.4	88.8	95.9
average	**91.9**	**96.8**	**94.6**	**89.0**	**95.9**
$dApp^{2-}$	93.7	97.0	97.4	94.0	91.4
$dCpp^{2-}$	92.9	97.5	96.0	93.8	91.5
$dGpp^{2-}$	94.1	96.7	97.9	93.7	90.7
$dTpp^{2-}$	93.1	97.0	96.7	93.5	91.5
average	**93.4**	**97.0**	**97.0**	**93.8**	**91.3**
rApp	91.2	85.3	95.2	90.1	97.1
rCpp	90.9	86.6	94.9	89.8	95.1
rGpp	91.6	84.7	94.3	90.1	95.7
rUpp	92.8	86.6	93.9	90.8	95.3
average	**91.6**	**85.8**	**94.6**	**90.2**	**95.8**
$rApp^{2-}$	92.9	86.3	92.3	93.3	91.2
$rCpp^{2-}$	92.1	87.3	92.6	93.9	91.4
$rGpp^{2-}$	93.0	86.1	93.1	92.5	91.2
$rUpp^{2-}$	92.3	86.2	92.4	93.6	91.3
average	**92.6**	**86.5**	**92.6**	**93.3**	**91.3**

$\Delta G_s(A^\bullet)$, and $\Delta G_s(H^\bullet)$ (solvation free energies of AH, $A^\bullet$, and $H^\bullet$). Herein, ΔG_{gas} can be easily calculated from the gas-phase BDE and the standard entropy correction terms. The solvation energies $\Delta G_s(AH)$ and $\Delta G_s(A^\bullet)$ can be calculated by the standard CPCM (conductor-like polarizable continuum model) method at the B3LYP/6-31G(d) level with water as the solvent.[12] The last term, $\Delta G_s(A^\bullet)$, equals 3.23 kcal/mol in aqueous solutions as previously estimated by Garrett using the path integral method.[19]

The results of the solution-phase calculations are listed in Table 3. From Table 3 it can be seen that for each particular position, the C−H bond dissociation free energy does not change much through the nucleobase change. To ease the following discussion, we have calculated the average C−H bond dissociation free energy for each position (see Table 3).

3.4. Comparison to the Previous Theoretical Predictions. To compare our present results with the previous theoretical predictions, we define the $C1'$−H bond dissociation free energy (or enthalpy) as the zero energy level and calculate the relative C−H bond strengths at the remaining positions (see Figures 4 and 5). As seen from Figure 4, when different model compounds are used, one can obtain dramatically different orders of C−H bond strengths for DNA-related radicals. For the simplest model compound (i.e. **1**), the C−H bond strengths decrease in the order $C2'$−H > $C4'$−H > $C3'$−H > $C1'$−H as predicted by both the ROHF/3-21G and MP2/6-31G* methods.[3,4] When neutral phosphate groups are introduced at 3′-OH and 5′-OH, the bond strengths decrease in the order $C2'$−H > $C3'$−H > $C5'$−H > $C4'$−H > $C1'$−H for model compound **2**.[4] When the NH_2 group of **2** is changed to a nucleobase, the bond strengths in the resulting compound (**3**) decrease in the order $C2'$−H > $C3'$−H > $C1'$−H > $C5'$−H > $C4'$−H.[6]

Our initial model compound (i.e., free deoxyribonucleoside, **4**) provides the order $C2'$−H > $C3'$−H ∼ $C4'$−H > $C5'$−H > $C1'$−H.[9] This order is fairly close to that predicted for model compounds **1** and **2**. However, when the two anionic phosphate groups are introduced (as in the negative pattern), the model compound **5** gives the order $C2'$−H ∼ $C3'$−H > $C4'$−H ∼ $C1'$−H > $C5'$−H. Furthermore, when two neutral phosphate groups are introduced (as in the neutral pattern), the model compound **6** gives the order $C2'$−H > $C5'$−H > $C3'$−H > $C1'$−H > $C4'$−H. Comparing all these models (i.e. **1**−**6**), it is clear that the $C2'$−H bond is always predicted to be the strongest. On the other hand, the relative strengths of the other C−H bonds vary considerably depending on the phosphorylation state and the ionization state of the phosphate groups.

The effect of phosphorylation on RNA-related radicals is shown in Figure 5. As shown in Figure 5, the initial nonphosphorylated model compound (**7**) gives the order $C3'$−H > $C2'$−H > $C4'$−H > $C1'$−H.[5] After addition of the actual nucleobase and 5′-OH to **7**, our own model compound (i.e. ribonucleoside, **8**) shows a fairly different order of C−H bond strength, which is $C3'$−H > $C1'$−H > $C2'$−H > $C4'$−H ∼ $C5'$−H.[9] Further addition of two anionic phosphate groups to **8** gives model compound **9**, whose C−H bond strength decreases in the order $C4'$−H > $C3'$−H ∼ $C1'$−H > $C5'$−H

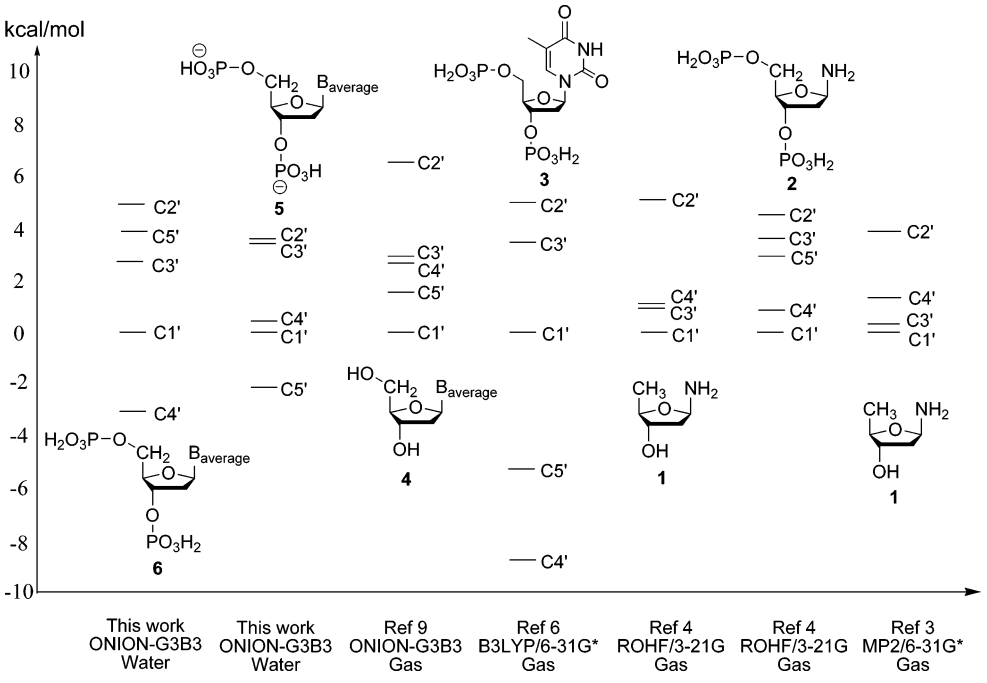


Figure 4. Comparison of relative stabilities of DNA sugar radicals predicted by using different model compounds.

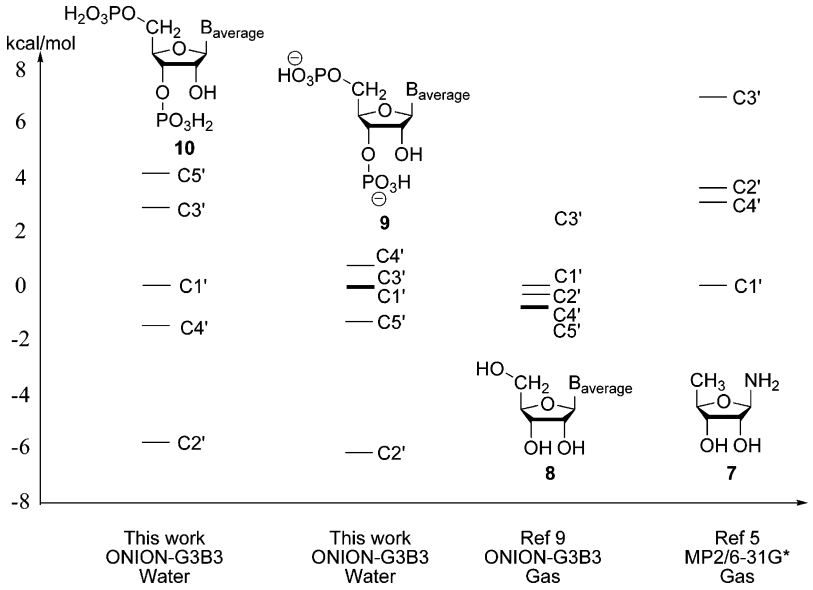


Figure 5. Comparison of relative stabilities of RNA sugar radicals predicted by using different model compounds.

≫ C2′−H. Finally, if the neutral phosphate group is added to **8**, the resulting compound **10** exhibits the order C5′−H > C3′−H > C1′−H > C4′−H ≫ C2′−H. Comparing all these model compounds, we conclude that the relative strengths of C1′−H, C3′−H, C4′−H, and C5′−H vary considerably depending on the phosphorylation state and the ionization state of the phosphate groups. Nonetheless, in all the model compounds there is a striking effect of phosphorylation associated with the strength of C2′−H. While C2′−H is only 0.2 kcal/mol weaker than C1′−H in compound **8**, C2′−H in both compounds **9** and **10** is about 6 kcal/mol weaker than C1′−H. This means that C2′−H should be a highly vulnerable site in phosphorylated RNA toward H-abstraction, regardless of the ionization state of the phosphate groups.

The strikingly low bond dissociation free energy of C2′−H in ribonucleoside 3′,5′-bisphosphate may be used to explain some previous experimental observations. For instance, it has been observed that nonspecific hydroxyl radical-induced strand breakage of polyuridylic acid was mainly caused by H-abstraction at the H-2′ position.[20] Furthermore, in the field of radiation damage of nucleic acids, it has been demonstrated that radiation damage of RNA is distinctly different from that of DNA. An initial damage to the base in RNA can be effectively transferred to the sugar mainly through H-2′ abstraction by the base radical.[21] However, such a mechanism is much less likely in DNA. All these experimental observations hint that the C2′−H bond of RNA is much more active than the other C−H bonds in RNA. This appears to be in excellent agreement with our theoretical predictions.

4. Discussion

By using a much more reliable theoretical method (i.e. ONION-G3B3) and by using much more suitable model compounds (i.e. nucleoside 3′,5′-bisphosphates) we have revised the predictions about the relative thermodynamic susceptibility of H-abstraction at each position of nucleic acids. Herein, we conduct further analyses in order to learn how phosphorylation brings about these interesting phenomena.

4.1. Effect of Phosphate Groups on Carbon Radical Stability. The effect of an α-substituent on the stability of carbon radicals has been studied extensively. It has been concluded that the α-substituent affects C−H BDE in four general ways:[8,22] (1) π-acceptors (e.g. CN, NO_2) reduce the C−H BDE because they delocalize the unpaired electron into their π-systems stabilizing the radical. (2) Lone-pair donors (e.g. NH_2, OH) reduce the C−H BDE because they stabilize the radical through a two-center three-electron interaction. (3) Alkyl groups reduce the C−H BDE because they stabilize the radical through hyperconjugation. (4) Polyfluoroalkyl groups increase the C−H BDEs because they destabilize the radical by inductively withdrawing electron density from the electron-deficient radical center.

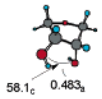
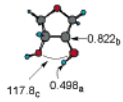
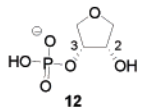
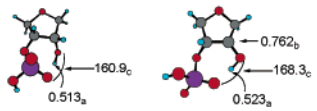
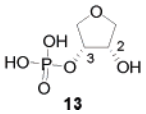
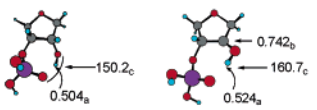
TABLE 4: The Effects of Phosphorylation on the C−H BDEs (kcal/mol)

Compound	3-Dimensional Structures		BDE (G3B3)
	Molecule	Radical[a]	
H−C−H			104.3
HO−C−H			96.3
HO−P(=O)(OH)−O−C−H			100.7
⊖O−P(=O)(OH)−O−C−H			95.0

[a] Each number indicates the spin density localized at a particular atom.

Despite the extensive studies in the past, no one has ever reported the phosphorylation effect on the stability of a carbon radical. Herein, we have calculated the C−H BDEs of four substituted methane molecules at the G3B3 level (see Table 4). As shown in Table 4, the OH, OPO_3H_2, and OPO_3H^- groups are all radical stabilization substituents because they reduce the C−H BDEs of methane. A further evidence for the radical stabilization ability of these groups is that they all reduce the spin carried by the carbon center. Nonetheless, compared to the OH group, the OPO_3H_2 group is less effective in delocalizing

TABLE 5: The C2−H BDEs of Model Compounds 11−13 (kcal/mol)

Compound	3-Dimensional structure[a]		BDE (ONIOM-G3B3)
	Molecule	Radical	
 **11**			96.6
 **12**			90.1
 **13**			92.3

[a] Each number refers to the natural orbital charge population (ending with "b"), the spin density (ending with "c"), and the O−H···O angle (ending with "d") at a particular atom or molecule.

the spin whereas the OPO_3H^- group is more effective. As a result, the C−H BDE of $CH_3-OPO_3H^-$ is 1.3 kcal/mol lower than that of CH_3OH, whereas the C−H BDE of $CH_3-OP_3H_2$ is 4.4 kcal/mol higher than that of CH_3OH.

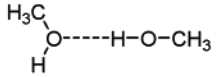
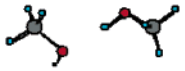
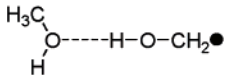
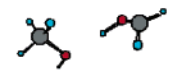
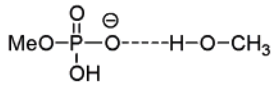
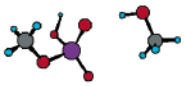
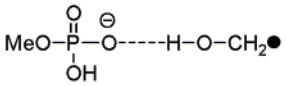
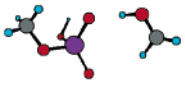
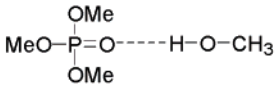
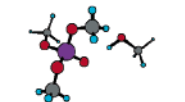
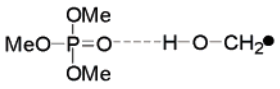
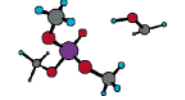
The results in Table 4 indicate that the radical stabilization effect of an *O*-phosphate group is different from that of an OH group. Furthermore, it is clear from Table 4 that the effect of a neutral OPO_3H_2 group is dramatically different from that of an anionic OPO_3H^- group. As a result, the relative strengths of the C−H bonds in DNA and RNA may vary considerably depending on the phosphorylation state and the ionization state of the phosphate group. This may explain why very different patterns of hydrogen abstraction have been observed in the radical-mediated DNA and RNA lesions under different conditions, because some subtle change in the experimental conditions (in particular, salt concentration and pH value) may lead to dramatically different orders of C−H bond strengths.

Nonetheless, it must be stressed that two particular bonds are not significantly affected by phosphorylation. The first is the C2′−H bond of DNA, which is always the most difficult site for hydrogen abstraction. The second is the C2′−H bond of RNA which, as discovered in the present study for the first time, is always the most vulnerable site for hydrogen abstraction regardless of the ionization state of the phosphate group.

4.2. Concerning the C2′−H Bond of Ribonucleotides. Because ribonucleoside 3′,5′-bisphosphate is a fairly complex molecule, we decided to use much simpler compounds (i.e. **11**−**13**) to investigate the extraordinary phosphorylation effect on C2′−H BDE. Due to the relatively simpler structures of these model compounds, we are able to rigorously screen the entire potential energy surfaces of these two molecules (each has 10 possible envelop conformations and 20 possible twist conformations) and find the global minimum structures for both the parent molecules and the resulting radicals (see Table 5). Using these global minimum structures we calculate that the C2−H BDE of **11** is 96.6 kcal/mol, the C2−H BDE of **12** is 90.1 kcal/mol, and the C2−H BDE of **13** is 92.3 kcal/mol. These values confirm the striking prediction that the C2′−H of ribonucleoside 3′,5′-bisphosphate is extraordinarily low, regardless of the ionization state of the phosphate group.

Further analyses of Table 5 reveal the following important observations. First, the O2−H bond in compound **11** can form a hydrogen bond with the neutral O3 atom via a five-membered ring. The O−H···O angle in the radical form of **11** is 117.8°.

TABLE 6: Hydrogen Bonding Interactions (kcal/mol) Calculated at the MP2/6-311++G(3df,3pd)//MP2/6-31+G(d) Level

Interacting species	3-Dimensional Structure	ΔE^a
		-3.7
		-4.1
		-14.1
		-17.0
		-6.6
		-7.1

[a] Hydrogen bonding interaction energy is corrected with BSSE.

On the other hand, the O2−H bond in compounds **12** and **13** can form a hydrogen bond with an oxygen atom in the 3-phosphate group via a seven-membered ring. The O−H···O angles in the radical form of **12** and **13** are 168.3° and 160.7°, respectively. Second, in the radical form of **11** the spin density at the carbon center is 0.822, while in the radical form of **12** and **13** the spin densities at the carbon center are 0.762 and 0.742, respectively.

The above observations connect the extraordinarily low C2−H BDE of **12** and **13** to the hydrogen bonding interactions of the 2-OH group. To gain further insights about such hydrogen bonding interactions, we have calculated the complexes of methanol and methanol radical with methanol or organic phosphate (see Table 6). According to Table 6, the strengths of the hydrogen bonding of methanol with methanol and methanol radical are 3.7 and 4.1 kcal/mol, respectively. These values indicate that the hydrogen bonding between two neutral OH groups is not strong. Furthermore, the optimal O−H···O angle in the hydrogen bonds is close to 180°, which is a value significantly higher than the 117.8° observed in the radical form of **11**. As a result, the hydrogen bonding energy between 2-OH and 3-OH in **11** should be close to zero.

On the other hand, the strengths of the hydrogen bonding of methanol and methanol radical with anionic or neutral phosphates are 14.1−17.0 and 6.6−7.1 kcal/mol, respectively. Thus the hydrogen bonding between OH and a phosphate group is much stronger than that between two hydroxyl groups. Furthermore, it is worth noting that from **12** (or **13**) to its C2 radical the O−H···O angle increases from 160.9° to 168.3° (or from 150.2° to 160.7°), a value that is closer to the optimal value (i.e. about 180° as seen in Table 6). As a result, we expect a significant increase in the hydrogen bonding strength from **12** or **13** to its C2 radical. Due to the strengthening of the hydrogen bonding interaction, the energy of the C2 radical of **12** or **13** is dramatically lowered, resulting in the extraordinarily low C2−H BDE. We assume that the same mechanism also applies to ribonucleoside 3′,5′-bisphosphates.

5. Conclusion

The roles of nucleic acid radicals in the DNA and RNA damage cannot be properly understood in the absence of knowledge of the C−H bond strengths depicting the energy cost to generate each of these radicals. However, the previous theoretical studies on the relative energies of different nucleic acid radicals are not fully convincing mainly because of the use of oversimplified model compounds. In the present study we chose nucleoside 3′,5′-bisphosphates as model compounds for DNA and RNA, in which the effects of both the nucleobase and phosphorylation are taken into consideration. Using the newly developed ONIOM-G3B3 methods, we have calculated the gas-phase bond dissociation enthalpies and solution-phase bond dissociation free energies of all the carbohydrate C−H bonds in the model compounds. On the basis of these data we have the following new and important findings.

(1) The monoanionic phosphate group (OPO_3H^-) is a better radical stabilization group than the OH group by 1.3 kcal/mol, whereas the neutral phosphate group (OPO_3H_2) is a significantly worse radical stabilization group than OH by 4.4 kcal/mol. Due to these reasons, the relative thermodynamic susceptibility of H-abstraction from deoxyribonucleotides and ribonucleotides varies considerably depending on the phosphorylation state and the charge carried by the phosphate groups.

(2) Strikingly, the bond dissociation free energy of C2′−H in ribonucleotides is dramatically lower than that of all the other C−H bonds by 5−6 kcal/mol regardless of the phosphorylation state and the charge carried by the phosphate group. This explains the previous experimental finding that radiation damage of RNA occurs mainly via H-abstraction at H-2′. A model study suggests that the strength of the hydrogen bonding interaction between the 2′-OH and 3-phosphate groups should dramatically increase from ribonucleoside 3′,5′-bisphosphate to its C2′ radical. The strengthened hydrogen bonding stabilizes the C2′ radical, rendering the C2′−H bond of RNA extraordinarily vulnerable to H-abstraction.

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Supporting Information Available: Detailed optimized geometries, core layer divisions for nucleotides, and detailed energies. This material is available free of charge via the Internet at http://pubs.acs.org.

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