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Damage to Model DNA Fragments from Very Low-Energy (<1 eV) Electrons

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Abstract: Although electrons having enough energy to ionize or electronically excite DNA have long been known to cause strand breaks (i.e., bond cleavages), only recently has it been suggested that even lower-energy electrons (most recently 1 eV and below) can also damage DNA. The findings of the present work suggest that, while DNA bases can attach electrons having kinetic energies in the 1 eV range and subsequently undergo phosphate−sugar O−C σ bond cleavage, it is highly unlikely (in contrast to recent suggestions) that electrons having kinetic energies near 0 eV can attach to the phosphate unit's P=O bonds. Electron kinetic energies in the 2−3 eV range are required to attach directly to DNA's phosphate group's P=O π^* orbital and induce phosphate−sugar O−C σ bond cleavages if the phosphate groups are rendered neutral (e.g., by nearby counterions). Moreover, significant activation barriers to C−O bond breakage render the rates of both such damage mechanisms (i.e., P=O-attached and base-attached) slow as compared to electron autodetachment and to other damage processes.

I. Introduction

There has been considerable recent interest[1] in the fact that low-energy electrons (i.e., electrons below ionization or electronic excitation thresholds) have been observed to damage DNA and in the mechanisms by which this can occur.

After summarizing several very recent observations and predictions,[1−6] we present our new theoretical results aimed at addressing the possibility that electrons may attach directly to phosphate groups and induce bond cleavage. Our findings lead us to conclude that this attachment process is very slow and occurs following a considerably different process than that described in ref 6. After reviewing past observations (in section I), we present our new findings in section II and summarize all of the findings in section III.

A. Experiments with Electrons having $E > 3.5$ eV. This group's involvement in the study of how low-energy electrons may damage DNA was nurtured by beautiful experiments from Boudaiffa et al.[2] who observed single strand breaks (SSBs) to occur in DNA[7] when electrons having kinetic energies as low as 3.5 eV were used to irradiate their samples.

The existence of peaks in the plots of SSB yield versus electron kinetic energy, combined with earlier knowledge from the Burrow group of the energies[8] at which DNA's four bases' π^* orbitals attach electrons, lead the authors of ref 2 to suggest that the SSBs likely occur by formation of a metastable resonance anion state. That is, the incident electron is captured to form an anion that likely involves occupancy of a base π^* orbital, after which some bond (n.b., in ref 2 it is not determined which bond beaks) is ruptured to cause the SSB.

Because the SSB peaks occurred at energies (>3.5 eV) considerably above the lowest base π^* anion state energies, ref 2 suggested that so-called core-excited resonances are likely involved. These resonances involve, for example, attaching an electron to a π^* orbital and simultaneously exciting another electron from a π to a π^* orbital. The fact that such core-excited states usually have longer lifetimes (with respect to autodetachment) than do π^* shape resonances (in which an electron is simply attached to a π^* orbital) was also cited[2] as a reason that these states might be involved in SSB formation. That is, the longer lifetimes allow more time for bond rupture to occur.

B. Suggestion that Electrons with $E < 3.5$ eV Can Also Damage DNA. It thus appeared that electrons with energies >3.5 eV could attach to DNA bases and induce SSBs. However, which bonds are broken in the SSBs and the details of the

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(1) The subject was the topic of a recent popular science article: Collins, G. P. Fatal Attachments: Extremely low energy electrons can wreck DNA. *Sci. Am.* **2003**, September issue, 26−28.

(2) Boudaiffa, B.; Cloutier, P.; Hunting, D.; Huels, M. A.; Sanche, L. *Science* **2000**, *287*, 1658−1662.

(3) Barrios, R.; Skurski, P.; Simons, J. *J. Phys. Chem. B* **2002**, *106*, 7991−7994.

(4) Berdys, J.; Anusiewicz, I.; Skurski, P.; Simons, J. *J. Phys. Chem.*, in press.

(5) Hanel, G.; Gstir, B.; Denifl, S.; Scheier, P.; Probst, M.; Farizon, B.; Farizon, M.; Illenberger, E.; Märk, T. D. *Phys. Rev. Lett.* **2003**, *90*, 188104-1−4.

(6) Li, X.; Sevilla, M. D.; Sanche, L. *J. Am. Chem. Soc.* **2003**, *125*, 13668−13669.

(7) The DNA samples used in ref 2 were quite dry and contained only their structural water molecules. Moreover, their phosphate groups likely had countercations closely bound to them because the samples did not possess net positive or negative charges. It is very important to keep in mind that all of the experimental and theoretical data discussed in this paper relate to such neutral samples in which the phosphate groups do not possess negative charges prior to electron attachment.

(8) Aflatooni, K.; Gallup, G. A.; Burrow, P. D. *J. Phys. Chem. A* **1998**, *102*, 6205−6207.

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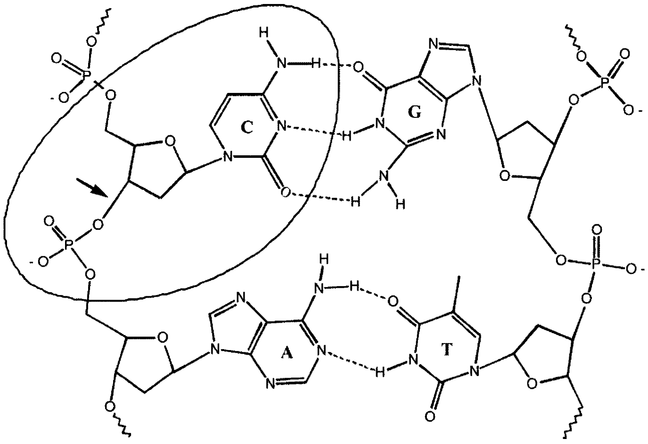

Figure 1. Fragment of DNA excised for study in refs 3,4 showing the bond that ruptures.

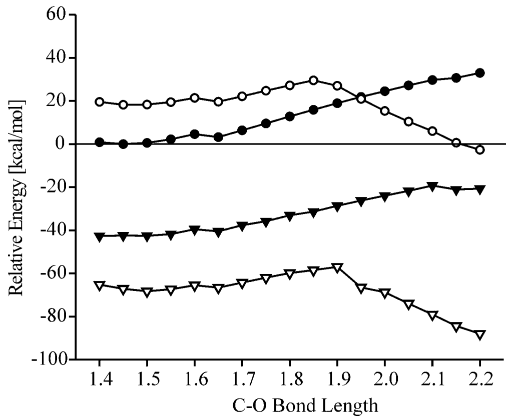

Figure 2. Energies of neutral (filled symbols) and anionic (open symbols) DNA fragments versus C−O bond length (Å) as isolated species (top two plots) and with $\epsilon = 78$ (bottom two plots). Reprinted with permission from ref 4. Copyright 2004 American Chemical Society.

mechanism of bond rupture were not yet resolved. We therefore undertook two theoretical studies[3,4] in which we excised[9] a base−sugar−phosphate unit (shown in Figure 1) of DNA and used theoretical simulations to further probe these matters.

We chose a cytosine-containing fragment because cytosine and thymine have the lowest-energy π^* orbitals, and we decided to consider whether even lower energy electrons than those studied in ref 2 might also induce SSBs. That is, we did not even focus on the core-excited (>3.5 eV) electron-attachment processes thought to be operative in ref 2. We instead proceeded to consider, for the first time to our knowledge, whether even lower energy electrons could cause SSBs by attaching to DNA's bases. In particular, we considered what happens when an electron is attached to a base π^* orbital (of cytosine in our simulations) because the experimental evidence clearly shows that such events can occur at energies below 3.5 eV (even below 1 eV for cytosine and thymine).

The primary findings of our earlier studies are summarized in Figure 2 and Table 1. In Figure 2, we plot the energy of the cytosine−sugar−phosphate fragment as the phosphate−sugar O−C bond is stretched[10] both in the absence of the attached electron and with an electron attached to cytosine's lowest π^* orbital. We plot these data both for an isolated (i.e., nonsolvated) fragment as is representative of the samples in ref 2 and when solvated by a medium characterized by a dielectric constant ϵ of 78. We performed the solvated-fragment simulations to gain some idea of how large an effect solvation might have on the SSB formation process we were considering.

Table 1. Barriers (kcal mol^{-1}) along the C−O Bond Length for Various Electron Kinetic Energies E (eV) and Various Solvent Dielectric Constants ϵ (from Ref 4)

	electron energy E (eV)					
	0.2	0.3	0.8	1.0	1.3	1.5
barrier ($\epsilon = 1.0$)	15.6	15.1	12.1	11.25	9.0	8.38
barrier ($\epsilon = 4.9$)	18.3	18.5	13.1	10.47	10.2	7.95
barrier ($\epsilon = 10.4$)	19.0	19.8	13.7	10.51	10.5	8.38
barrier ($\epsilon = 78$)	28.1	21.8	11.3	9.5	5.3	5.1

The crucial observation to make is that the anion surface has a barrier near 1.9 Å and subsequently drops to lower energy as R is further increased, while the neutral-fragment surface monotonically increases with R indicative of homolytic cleavage of the C−O bond. The barrier on the anion surface and its physical origin play central roles in the mechanism of SSB formation that we introduced in ref 3 as we discuss further later.

We carried out such simulations for a range of energies E for the electron that attaches to the π^* orbital because these metastable π^* anion states have substantial Heisenberg widths that derive from their short lifetimes. We varied the electron energy E to span the reasonable range of these widths. For each E value, we carried out simulations with the cytosine−sugar−phosphate unit surrounded by a dielectric medium of various solvation strengths (as characterized by the dielectric constant ϵ in the polarized continuum model (PCM) of solvation[11]). In Table 1, we summarize how the barrier on the anion surface depends on the electron energy E and the solvent dielectric strength ϵ.

We estimated the rates of C−O bond breakage, and thus of putative SSB formation, by taking the frequency at which a typical C−O bond vibrates (ca. 10^{13} s^{-1}) and multiplying by the probability P that thermal motions can access the barrier height Δ: $P = \exp(-\Delta/kT)$. It was thus suggested in refs 3,4 that accessing the barrier on the π^* anion surface would be the rate-limiting step in SSB formation by the π^* base anion mechanism that we suggested. Further, it was noted that barrier heights of 5, 10, 15, 20, and 25 kcal mol^{-1} are predicted (using rate $= 10^{13} \exp(-\Delta/RT)$ s^{-1}) to produce C−O rupture rates of 6.3×10^9, 1.3×10^6, 2.7×10^2, 6×10^{-2}, and 1×10^{-5} s^{-1}, for conditions (i.e., $\epsilon = 1$) most relevant to the experiments of ref 2. For example, when a 1 eV electron attaches to cytosine, the barrier height is 11 kcal mol^{-1}, and we predict SSBs involving phosphate−sugar O−C σ bond cleavage occur at ca. 10^6 s^{-1}. Because the rate of electron autodetachment from the

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(9) We terminated with H atoms the −O radical centers formed by excising the fragment shown, and we rendered neutral (by protonation) the negative charge shown in Figure 1 on the phosphate O atom to simulate the presence of the nearby countercation that no doubt is present in the dry samples of ref 2.

(10) We focused on this particular bond because it was clear to us that its rupture would be thermodynamically favored because of the large (ca. 4 eV) electron affinity of the phosphate unit that is formed upon its cleavage. However, as we discuss later, recent experimental data also suggest that other bonds within the bases themselves may also be subject to cleavage. We recently considered cleavage of the sugar−base C−N σ bond when an electron is attached to the base (cytosine) π^* orbital and found the barrier to this bond rupture to be ca. 40 kcal mol^{-1}. We plan to continue such studies of other bond ruptures further in future work.

(11) Miertus, S.; Tomasi, J. *Chem. Phys.* **1982**, *65*, 239−242. Cossi, M.; Barone, V.; Cammi, R.; Tomasi, J. *Chem. Phys. Lett.* **1996**, *255*, 327−335.

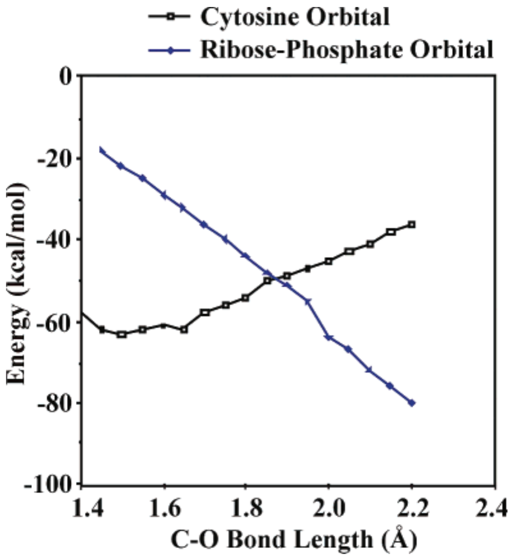

Figure 3. Plots of the π^* (lower curve at left) and σ^* (upper curve at left) diabatic anion energy states as functions of the C−O bond length R.

π^* anion state is ca. $10^{13}-10^{14}$ s^{-1}, this suggests that only 1 in ca. 10^7-10^8 such π^* anions will undergo SSB.

C. Mechanism for σ^* Bond Cleavage When π^* Anions Are Formed. Understanding the behavior of the anion-surface plots and the origin of the barriers on them proved crucial to understanding the mechanism proposed in refs 3,4.

The barrier is caused by an avoided crossing between a pair of diabatic anion states: (i) One state has the excess electron attached to the base π^* orbital and a pair of electrons occupying the phosphate−sugar O−C σ bonding orbital, and (ii) the second state has no electron in the base π^* orbital, two in the O−C σ orbital, and one electron in the O−C σ^* orbital.

An example of two such diabatic curves as functions of the C−O bond length is given in Figure 3.

In the absence of any coupling between these two diabatic states, the π^* state would lie above the neutral cytosine−sugar−phosphate curve by an amount E (i.e., the energy of the attached electron) and would parallel the neutral curve shown in Figure 2. That is, no barrier and subsequent drop off would be observed. In the region of the equilibrium C−O bond length (ca. 1.45 Å), the σ^* diabatic state is too high in energy to couple with the π^* state. However, as the C−O bond length R stretches to near 1.9 Å, the energy of this σ^* state drops (because the antibonding σ^* orbital becomes less antibonding as R increases) to an extent that brings its energy into resonance with that of the π^* diabatic state. In the region near $R = 1.9$ Å, the π^* and σ^* diabatic states can thus couple and the electron can migrate into the σ^* orbital thus initiating fragmentation of the C−O bond. Beyond $R = 1.9$ Å, the energy of the lowest adiabatic anion state, which has evolved into primarily σ^* character, drops to even lower energy because of the large electron affinity of the neutral phosphate group[9] formed when the C−O bond is fully ruptured and the sugar radical is formed. It should be noted that the suggested involvement of $\pi^*-\sigma^*$ coupling is not new; it has been shown[12] to be operative in many experimental studies.

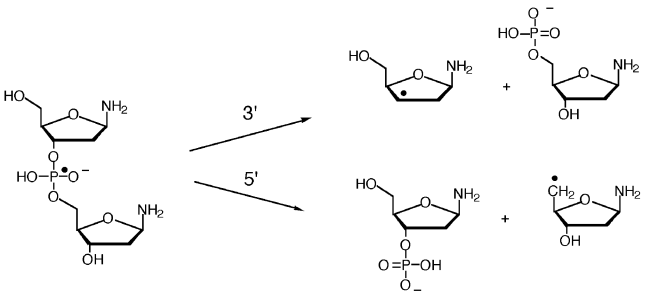

Figure 4. Fragmentation of 3′ or 5′O−C σ bonds in phosphate-centered anions. Reprinted with permission from ref 6. Copyright 2003 American Chemical Society.

In summary, our earlier work suggests that electrons in the 1 eV range can attach to base π^* orbitals and induce phosphate−sugar O−C σ bond cleavage, but that only one in ca. 10^7-10^8 such π^* anions do so. That work was the first time such low-energy electrons were predicted to cause such SSBs via this mechanism.

D. Experiments on Uracil Showing σ Bond Cleavage for Electrons near 1 eV. Recently, an experimental study[5] on gas-phase samples of uracil $C_4N_2O_2H_4$ (thought to be reasonably representative of DNA bases' π^* orbital structure) showed that electrons with energies near 1 eV can induce H atom loss to produce $C_4N_2O_2H_3^-$ anions. At higher electron energies, other bond cleavages also occur to generate other anion fragments.

The data obtained in ref 5 suggest[13] that ca. one in 10^4-10^5 uracil anions undergo H atom loss; the remaining anions undergo autodetachment, so the rates of H atom loss we estimate to be ca. 10^5 s^{-1} or more. These data are similar to or somewhat larger than the branching ratios predicted for C−O bond rupture in our study of the cytosine−sugar−phosphate model system discussed earlier, but, of course, in this study it is a bond to an H atom that is broken. Nevertheless, these data certainly motivate us to consider other bond cleavages in our base−sugar−phosphate studies, as we plan to do.

E. Theoretical Suggestion that Even Zero-Energy Electrons May Attach Directly to Phosphate Units. Even more recently, another theoretical study was carried out[6] in which anions of sugar−phosphate−sugar′ units were examined, and the findings were used to suggest that low-energy electrons may attach directly to phosphate groups and induce SSBs by fragmenting 3′ or 5′C−O σ bonds as illustrated in Figure 4. It should be noted that, prior to electron attachment, the sugar−phosphate−sugar′ (which we denote SS′(OH)P=O) is neutral, not negatively charged much as is the phosphate group in our phosphate−sugar−base study.

The findings of ref 6 suggest that rather than attaching to a DNA base π^* orbital as we had originally suggested,[3,4] alternatively it may be possible for a very low-energy electron to attach directly to the phosphate moiety to form a $P^\bullet-O^-$ radical anion which might live long enough to subsequently induce rupture of a 3′ or 5′P−O σ bond.

Plots of the SS′(OH)P=O neutral and SS′(OH)$P^\bullet-O^-$ anion surfaces along the 3′ and 5′C−O bond lengths from ref 6 are shown in Figure 5 for the model compounds shown in Figure 4.

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[12] For example, see: Pearl, D. M.; Burrow, P. D.; Nash, J. J.; Morrison, H.; Nachtigallova, D.; Jordan, K. D. *J. Phys. Chem. A* **1995**, *99*, 12379−12381.

[13] We reach this rough estimate using 10^{-16} cm^2 as the effective cross section for a uracil-electron collision, which likely is a lower bound to this quantity. Hence, our estimate that one in 10^4-10^5 anions lose an H atom is an upper bound.

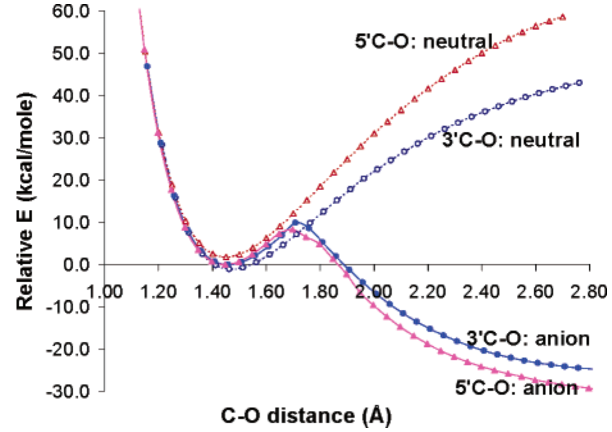

Figure 5. Plots of the neutral and anion surfaces as functions of C−O bond lengths for the fragments examined in ref 6. Reprinted with permission from ref 6. Copyright 2003 American Chemical Society.

It is important to note that the mechanism by which an electron attaches to the SS′(OH)P═O to form the P$^\bullet$−O$^-$ radical anion was not discussed in ref 6. We emphasize this because it is in the present work that we offer what we believe is the most likely interpretation of such an event. However, in ref 6, the conclusion was rendered that, once such a radical anion is formed, subsequent fragmentation of either the 3′ or the 5′C−O σ bond would require surmounting a ca. 10 kcal mol^{-1} barrier on the anion surfaces as shown in Figure 5.

Although it was not discussed in ref 6, we think the form of the anion curves shown in Figure 5 is reminiscent of the avoided-crossing induced curves shown in Figure 2 in which a descending σ^* anion diabatic state crosses a π^* diabatic state. In the SS′(OH)P═O case, we suggest the latter diabatic state is likely one in which the P═O double bond in the neutral parent compound has had an electron attached to its π^* orbital, thus breaking the π bond and producing a P$^\bullet$−O$^-$ anion radical. The other diabatic state would then be one in which the P═O double bond is intact but the excess electron is in an O−C σ^* orbital involving either the 3′ or the 5′ bond. The avoided crossing between these two diabatic states then allows the excess electron to migrate from the P$^\bullet$−O$^-$ center onto the C−O bond that breaks.

In summary, the theoretical study of the SS′(OH)P═O model system predicts that very low-energy electrons (even 0 eV electrons are mentioned as possibilities) can attach directly to the phosphate unit and subsequently break a phosphate−sugar O−C bond. This, of course, suggests that DNA damage may not require addition of an electron to a base π^* orbital but might utilize the phosphate unit directly. It was this suggestion that caused us to examine the phosphate-attachment process in more detail as we now describe. In anticipation, we mention that our findings indicate that the pictures of the SS′(OH)P═O neutral and SS′(OH)P$^\bullet$−O$^-$ anion energy surfaces shown in Figure 5 are not entirely correct and must be modified in the $R < 1.7$ Å region where the anion states are actually electronically metastable rather than stable as suggested in Figure 5.

It should be emphasized that our findings are not simply a refinement of the earlier work of ref 6. Instead, they imply that 0 eV electrons will not attach directly (i.e., vertically) to the phosphate units with any facility. Rather, direct attachment can produce a metastable P═O π^* anion but would require electrons with >2 eV kinetic energy. In addition, for the nascent P═O π^* anion to undergo bond rupture, a barrier arising from a $\pi^*-\sigma^*$ curve crossing must be surmounted. Let us now present these results in detail and discuss their implications.

II. Our Study of Direct Electron Attachment to Phosphate

A. What Special Methods Are Needed, and What Needs To Be Improved/Corrected? As noted above, we do not believe the description of the SS′(OH)P$^\bullet$−O$^-$ anion states as discussed in ref 6 and summarized in Figure 5 is qualitatively correct for R values smaller than 1.7 Å. Figure 5 shows the anions to be electronically stable in this region, but we believe the anions are metastable. In particular, on the basis of our experience with the cytosine−sugar−phosphate system, we anticipate it is essential to include two anion diabatic configurations in the description of the lowest adiabatic state and to consider the coupling between these diabatic states that thus generates the lowest adiabatic anion state. Any barrier in this adiabatic state and the energy and geometry where this adiabatic state's energy surface intersects the neutral's energy surface will play key roles in the electron-attachment and bond-rupture events.

Specifically, one diabatic configuration in which the excess electron is attached to the P═O π^* orbital (thus forming the P$^\bullet$−O$^-$ radical anion center) and another in which the excess electron occupies an O−C σ^* orbital must be included. We will denote these two configurations the π^* and σ^* anion states in the following discussion, and we will see that the π^* state is metastable for all R-values while the σ^* state is metastable at short R but stable at longer R.

The electronic energies of the π^* state and of the σ^* state in the small-R region cannot be treated using standard electronic structure methods. The use of straightforward variational ab initio techniques for such electronically unstable states even with extremely large basis sets is doomed to fail. Such approaches generate an anion wave function that describes the neutral molecule plus a free electron infinitely distant and with essentially zero kinetic energy.[14] This happens because the molecule-plus-free-electron function is indeed the lowest-energy solution to the Schrödinger equation in such cases; of course, it is not the solution we seek when we attempt to describe metastable resonance states of anions. The function we want is embedded within the continuum of molecule-plus-free-electron functions. Using smaller basis sets that exclude diffuse basis functions cannot easily avoid these difficulties; instead, one must employ an approach such as we now detail. Finally, to eliminate the possibility that our calculations produce (unwanted here) dipole-bound anion states, we simply do not employ the kind of extra diffuse basis functions (centered on the positive end of the molecule's dipole moment) needed to describe such states.

B. Methods We Use To Handle the Metastable Anion States. In the so-called stabilization method[15,16] that we

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(14) In our opinion, this is why the anion curves shown in Figure 5 are essentially parallel to the neutral curves for $R < 1.7$ Å. Of course, in ref 6 as in the current work, a basis of finite radial extend was used. Thus, it was not possible to obtain an anion state energy exactly equal to the energy of the neutral (i.e., to describe the "extra" electron infinitely distant and not moving). As a result, the anion's energy is only nearly equal to that of the neutral, and thus the "extra" electron resides at a finite (large) distance with finite kinetic energy.

(15) Hazi, A. U.; Taylor, H. S. *Phys. Rev.* **1970**, *A1*, 1109−1116.

(16) Simons, J. *Resonances in Electron−Molecule Scattering, van der Waals Complexes, and Reactive Chemical Dynamics*; ACS Symposium Series; American Chemical Society: Washington, DC, 1984, 3−16.

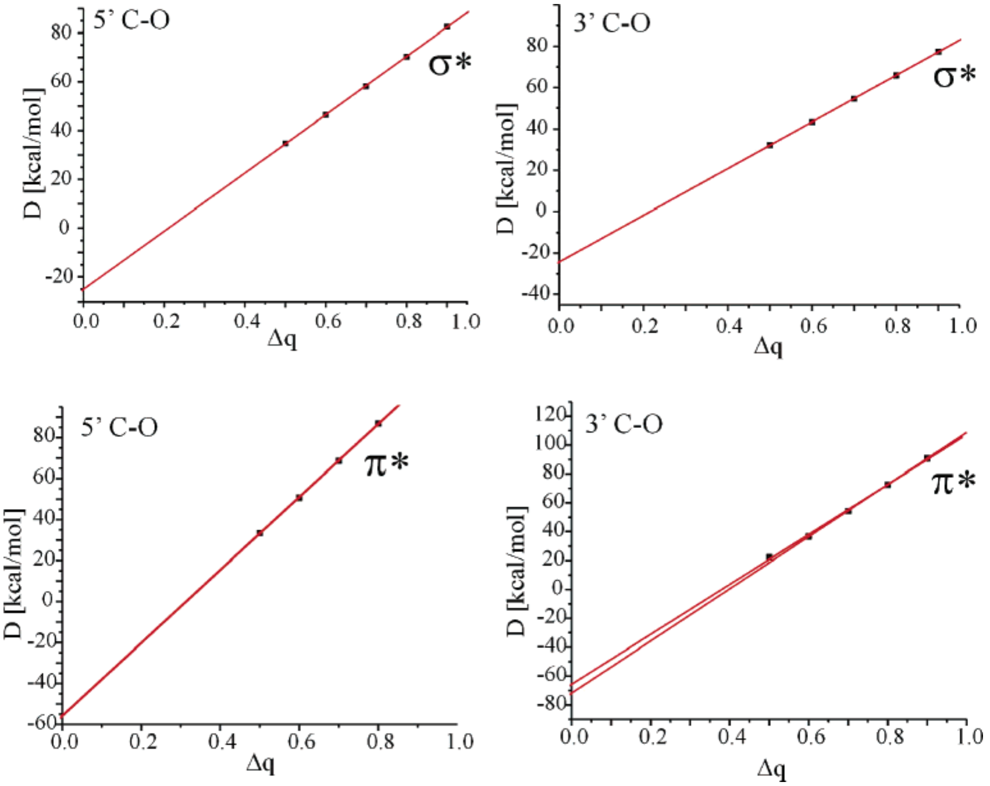

Figure 6. Stabilization plots for the 3′ and 5′C−O bond ruptures with the π^* (at 1.4 Å for the 3′ and 1.5 Å for the 5′) and σ^* (at 1.8 Å for both 3′ and 5′) diabatic states' SCF-level binding energies D plotted at values of the stabilizing charge Δq for which these anion states are stable and then extrapolated[17] to $\Delta q \to 0$. In the 3′C−O π^* plot, which displays considerable curvature, we show two linear fits.[17]

employed in the present work, one adds to the Hamiltonian H describing the SS′(OH)P═O + e$^-$ system of interest a potential V that confines the excess electron to the molecular framework (i.e., does not allow it to escape). One then carries out a series of calculations on the anion using $H + \lambda V$ as the Hamiltonian (using values of λ for which the electronic state of interest is bound and thus amenable to conventional electronic structure treatment) and determining the energy of the desired anion state for such a range of λ values. Because the energies of the neutral states are usually altered when the Hamiltonian is replaced by $H + \lambda V$, one must also compute their energies at various λ values. By then extrapolating the anion and neutral state energies to $\lambda = 0$, one obtains the stabilization estimate for these energies.

In the particular variant of the stabilization method used here to obtain the resonance-state energies for the π^* state and the σ^* state in the small-R region, we artificially increased the nuclear charge of the phosphorus atom by an amount Δq to render the anionic states electronically bound. For each value of the 3′ or 5′C−O bond length R (with all of the other geometrical parameters of the anion varied to minimize the energy of the neutral), these calculations were performed for several values of Δq, and the anion−neutral energy differences were then extrapolated to $\Delta q = 0$ to obtain the true energy of the anion relative to that of the neutral. Examples of such extrapolations are shown in Figure 6 where we plot the SCF-level electron binding energy D versus the incremental charge Δq for σ^* and π^* anions and for 3′ and 5′ bond cleavages.

It should be emphasized that we only use computed values of D that correspond to electronically stable anion states; that is, we do not include in our fitting any data for Δq values where the computed D values are negative. This is done because only when D is positive can the ab initio methods be "trusted" because only here is the anion electronically bound. We should also note that because the anion states lie significantly above the neutral at several R-values, the computed D values are positive only for rather large Δq. As a result, we are forced to extrapolate over a substantial range of Δq, thereby generating significant uncertainties in our predicted metastable-state anion energies. Below, we attempt to offer approximations to the uncertainties introduced by such extrapolations.

C. Our Neutral, π^*, and σ^* Anion Curves for 3′ and 5′ Bond Rupture. At all values of the phosphate−sugar O−C bond lengths for which the anion states are not electronically stable (i.e., not lower than the energy of the neutral), we carried out such stabilization extrapolations to obtain our final estimates of the anion states' electron binding energies. We performed these calculations at the self-consistent field (SCF) level using the same 6-31+G* atomic orbital basis sets that were shown to be adequate in our earlier studies[3,4] within the Gaussian 98 suite of programs[18] using our dual processor Athlon computers and those of the Utah Center for High Performance Computing. We should note that the calculations of ref 6 were performed using density functional theory (DFT) and thus include electron

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(17) The plots of D versus Δq were fit to first-order polynomials in Δq to effect the extrapolations. In cases where the data points displayed considerable "curvature" at small Δq values, we performed two fits. One first-order polynomial fit was carried out in which all of the data points are used, and another was carried out in which we eliminate the data points at small Δq where the curvature occurs. The quality of both the linear and the quadratic fits is high, with statistical r-values of 0.95 or greater in all cases. The justification for eliminating the data points at small Δq where curvature is present is that such behavior suggests that the diffuse functions in our basis are (incorrectly) causing the anion's energy to be lowered toward the neutral-plus-free-electron collapse that the stabilization method attempts to avoid.

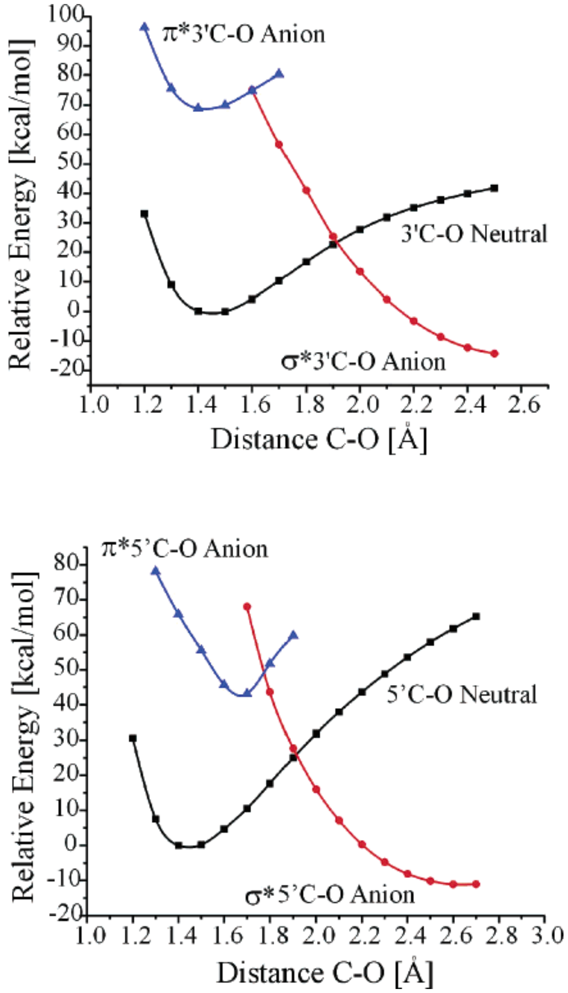

Figure 7. Energies[20] of neutral, π^* anion, and σ^* anion for 3′C−O (top) and 5′C−O (bottom) bond rupture versus C−O bond length.

correlation contributions to the total energy. As such, the results of ref 6 are intrinsically more accurate than ours[19] at R-values where the anions are indeed electronically stable. However, as we detailed above, only our results are qualitatively correct where the anion states are metastable.

In Figure 7, we show the neutral and π^* and σ^* diabatic anion curves obtained using the stabilization method discussed above for fragmentation of the 3′C−O and 5′C−O bonds, respectively. In Figure 8, we depict the representative π^* and σ^* molecular orbitals that are singly occupied in the corresponding anionic states.

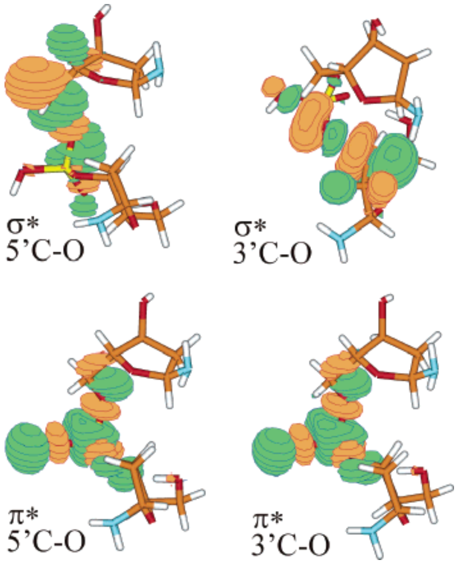

Figure 8. Examples of anionic singly occupied σ^* (top) and π^* (bottom) molecular orbitals computed at values of the stabilizing charge Δq of 0.7 au for which these anion states are stable. These MOs correspond to R = 1.8 Å for the σ^* states and R = 1.4 Å for the π^* states.

Although many of the features shown in Figure 7 are similar to those appearing in Figure 5, there are important differences that need to be discussed. First, because the data of Figure 5 were obtained using the correlated DFT-level treatment, they likely are more accurate[19] for large R-values where the anions are electronically stable. Thus, the neutral-anion energy gaps shown in Figure 5 at, for example, R = 2.5 Å (i.e., 60 kcal mol^{-1} for the 3′C−O bond fragmentation and 80 kcal mol^{-1} for 5′ bond fragmentation) are probably more accurate than our 55 and 70 kcal mol^{-1}, respectively. For these reasons, it is probable that our anion curves are too high, relative to our neutral curves, by ca. 5−10 kcal mol^{-1}. This observation is used later to justify the possibility of shifting our anion curves downward by 5−10 kcal mol^{-1} to arrive at a range of estimates for the energy barriers that may play crucial roles in the bond-rupture process.

The energies and R-values at which the lowest anion curve (i.e., the σ^* curve) crosses the neutral's energy curve obtained from Figure 7 are ca. 20−25 kcal mol^{-1} and 1.9 Å for both the 3′ and the 5′ cleavages. These values for the data of Figure 5 are ca. 10 kcal mol^{-1} and 1.7 Å. However, if we were to shift our anion curves in Figure 7 downward by 5−10 kcal mol^{-1}, our σ^* anion−neutral intersection energies and R-values would be very close to those shown in Figure 5. Hence, we believe that the geometry (R = 1.7 Å) and energy (10 kcal mol^{-1}) at which the σ^* anion state intersects the neutral state are correct as shown in Figure 5.

However, for R-values where the anion states are metastable, the descriptions of the anion states depicted in Figures 5 and 7 are qualitatively different, and we claim only those of Figure 7 are correct. Therefore, only by using Figure 7 are we able to suggest what can happen when very low-energy electrons strike neutralized (e.g., by nearby counterions) phosphate groups in DNA.

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(18) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, revision A.7; Gaussian, Inc.: Pittsburgh, PA, 1998.

(19) Although DFT methods are known to not accurately describe the electron−molecule interactions for large electron−molecule distances, they have been successfully used when describing bound states of anions in which the "extra" electron occupies a conventional valence orbital. For these reasons, we do not feel it appropriate to doubt the accuracy of the curves shown in Figure 5 at larger R-values.

(20) Whenever the stabilization plots displayed significant curvature at small Δq, we obtained two estimates of the $\Delta q \rightarrow 0$ electron binding energy. In Figure 7, we plot the average of these two linear-extrapolation values. In all cases, the estimates obtained using the two methods[17] differed by no more than 10 kcal mol^{-1}.

Specifically, our data suggest the following:

(1) Near the equilibrium geometry ($R = 1.4$ Å) of the neutral SS′(OH)P═O, 0 eV electrons will not attach to form SS′(OH)-P$^\bullet$−O$^-$; instead, electrons with energies near 2−3 eV (or 1.5−2.5 eV if we shift our anion curves downward as discussed above) are required to form the P═O π* anion.

(2) For that very small fraction (F) of the SS′(OH)P═O sample whose P═O bond is stretched by thermal vibrational excitation to near 1.7−1.9 Å ($F = \exp(-10\ \text{kcal mol}^{-1}/kT) = 4 \times 10^{-8}$ at $T = 298$ K), 0 eV electrons can indeed attach to the 3′ or 5′C−O σ* orbital to form a stable anion which subsequently fragments. However, the rate at which the neutral accesses the anion−neutral crossing point[21] will be approximately $10^{13} \exp(-10\ \text{kcal mol}^{-1}/kT) = 4 \times 10^{5}\ \text{s}^{-1}$ at $T = 298$ K. This then is our estimate of the maximum[22] rate at which 0 eV electrons could be attached to SS′(OH)P═O.

(3) For the majority of the SS′(OH)P═O sample molecules that are struck by a 2−3 eV electron while near $R = 1.4$ Å, a π* anion can be formed. However, this π* anion is metastable to autodetachment (at a rate of ca. $10^{14}\ \text{s}^{-1}$) and must surmount a barrier at which it couples with and evolves into the σ* anion state that can subsequently fragment. The barriers at which the π* and σ* curves cross occur at 1.9 Å and lie 7 and 10 kcal mol^{-1} above the nascent π* anion's minimum, respectively, for the 5′ and 3′ fragmentation cases. Using the average of these two barrier heights, the thermal probability P of surmounting such a barrier is predicted to be $P = \exp(-8.5\ \text{kcal mol}^{-1}/kT) = 5 \times 10^{-7}$ at $T = 298$ K, and the rate of such crossings[21] is $10^{13} \exp(-8.5\ \text{kcal mol}^{-1}/kT) = 5 \times 10^{6}\ \text{s}^{-1}$ at $T = 298$ K.

Finally, we note that neither the rate of bond cleavage induced by 0 eV electrons (ca. $4 \times 10^{5}\ \text{s}^{-1}$) nor the rates at which cleavage is caused by 2−3 eV electrons forming the P═O π* anion (ca. $5 \times 10^{6}\ \text{s}^{-1}$) will be very competitive with autodetachment (occurring at $10^{14}\ \text{s}^{-1}$) at $T = 298$ K. Only the latter rate seems to be comparable to the rates we estimated earlier[3,4] for C−O bond rupture induced by electrons captured into base π* orbitals. Of course, in living organisms, other damage mechanisms (e.g., oxidative damage, protonation, etc.) are also operative. On the basis of the very slow rates predicted in the present work for damage induced by direct attachment of low-energy electrons to P═O π* orbitals in neutralized phosphate units, we suggest that the electron-damage mechanism studied here is not likely to be a major contributor to overall DNA C−O bond rupture.

III. Summary

The results presented in this paper and findings of earlier workers,[2−6] on dry DNA samples, on base−sugar−phosphate model systems, on gas-phase uracil, and on model sugar−phosphate−sugar′ compounds, suggest the following:

(1) DNA bases likely[3,4] can attach electrons having kinetic energies even below 1 eV to their π* orbitals and undergo (neutralized) phosphate−sugar O−C σ bond cleavage at rates of ca. $10^{6}\ \text{s}^{-1}$ at $T = 298$ K.

(2) DNA bases may (as inferred from the uracil data[5]) attach 1 eV electrons and undergo loss of an H atom directly from the base's ring at rates of ca. $10^{5}\ \text{s}^{-1}$ or more.

(3) Electrons having kinetic energies near 0 eV cannot attach directly at significant rates (we estimate the rates to be $10^{5}\ \text{s}^{-1}$) to DNA's phosphate units (even if these units are rendered neutral by counterions) as deduced from the present work on SS′(OH)P═O model compounds.

(4) Electrons with energies in the 2−3 eV range can attach directly to DNA's (neutralized) phosphate group's P═O π* orbital and form a metastable π* anion. Such anions can induce phosphate−sugar O−C σ bond cleavages but only at rates of ca. $10^{6}\ \text{s}^{-1}$.

It thus appears that a variety of bond cleavages (e.g., phosphate−sugar O−C and base−H) can occur when low-energy electrons strike DNA whose phosphate groups are neutralized. Of course, it remains an important issue that none of the works discussed here addresses how often a particular phosphate group in a DNA molecule exists in such a neutralized state in living organisms. Because of the nature of the experimental conditions, the phosphate groups are so neutralized in the experiments and simulations that we discussed here. However, in living systems, the phosphates and their counterions exist in rapid-equilibrium conditions of association and dissociation. The relevance of our current findings thus may be that whenever counterions are close enough to make a particular phosphate unit neutral (even if for a brief timespan), that unit will be susceptible to the types of very low-energy electron-induced bond cleavages discussed here and likely base π* capture will be more important than P═O π* capture.

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[21] The rate we estimate by multiplying the probability P by the frequency at which the C−O bonds vibrate, the latter being ca. $10^{13}\ \text{s}^{-1}$.

[22] This estimate is an upper bound because it assumes an electron will indeed attach if it encounters a neutral at this stretched-R geometry.