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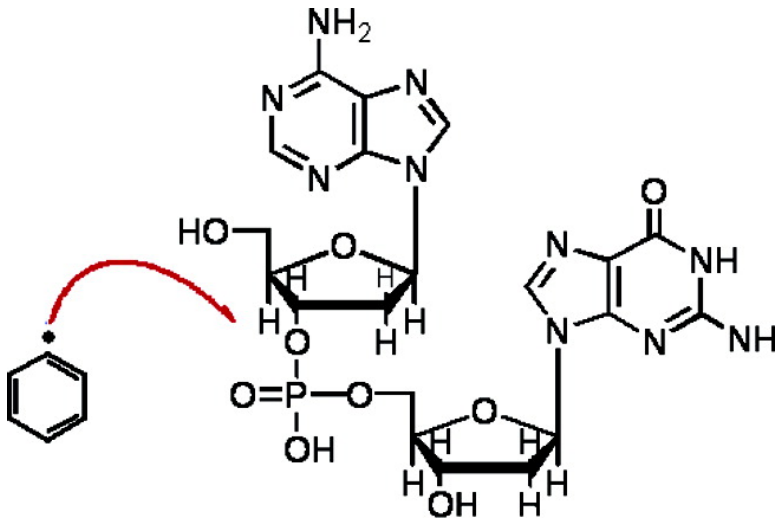

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Phenyl Radicals React with Dinucleoside Phosphates by Addition to Purine Bases and H-Atom Abstraction from a Sugar Moiety

Ji-ang Liu, Christopher J. Petzold,† Luis E. Ramirez-Arizmendi,‡ James Perez,§ and Hilkka Kenttämaa*

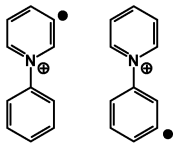
Department of Chemistry, Purdue University, 560 Oval Drive, West Lafayette, Indiana 47907-2038

Received April 28, 2005; E-mail: hilkka@purdue.edu

Nonhydrolytic DNA cleavage is known to occur upon the exposure of DNA to aromatic mono- and biradicals with carbon-centered σ-radical sites.[1] Therefore, efforts have been directed toward the understanding of the reactivity of phenyl radicals toward DNA components in solution.[1a,f,2,3] However, these studies are complicated by the high reactivity of the phenyl radicals. To the best of our knowledge, only one unambiguous study has been published on the reactions of phenyl radicals with DNA components in solution[1a] (the identity of the attacking radical is ambiguous in the few other papers published on this topic[1f,3]). Therefore, many fundamental questions remain unanswered. For example, it is currently not known whether DNA damage caused by a phenyl radical is initiated by attack at a sugar moiety or at a base (some evidence points to these two reactions as being competitive[4]), whether different bases have varying susceptibilities to phenyl radical attack, and whether the site of attack depends on the phenyl radical's exact structure.

In the past, we have employed mass spectrometry to examine the reactivity of phenyl radicals in the gas phase.[5] The phenyl radicals carry a chemically inert charged group to allow mass spectrometric manipulation. These studies have revealed that charged radicals yield the same products in reactions with simple organic substrates and small biomolecules (e.g., sugars, nucleobases, thymidine) as has been reported[1a] for neutral phenyl radicals in solution. Unfortunately, these gas-phase studies have been limited to small molecules that can be evaporated into the mass spectrometer by thermal heating. We recently demonstrated that laser-induced acoustic desorption (LIAD) can be used to evaporate thermally labile biomolecules into the gas phase as intact neutral molecules.[5c,6] We report here the first study on the reactions of phenyl radicals with dinucleoside phosphates evaporated by LIAD (Scheme 1). The

Scheme 1



a **b**

results demonstrate that phenyl radicals can attack both the sugar and the base moiety in these substrates, that purine bases are more susceptible to phenyl radical attack than pyrimidine bases, and that the structure of the attacking radical can have a major influence on these reactions.

The generation and isolation of the charged phenyl radicals in a dual-cell Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR) was carried out as described in the literature.[5] The dinucleoside phosphate samples (Sigma-Aldrich; >99.5% purity) were electrospray-deposited on 12 μm thick titanium foils, which were introduced into the instrument as described previously.[5c,6] The side of the foil that was not coated with sample was exposed to laser pulses that desorbed the neutral dinucleoside phosphates from the opposite side of the foil into the mass spectrometer. The ability of LIAD to evaporate intact neutral peptides into a mass spectrometer was demonstrated earlier,[5c,6] but this does guarantee that the experiment is equally successful for dinucleoside phosphates due to their greater sensitivity for thermal degradation. Therefore, dApdG was evaporated via LIAD into a clean glass vial and then analyzed by electrospray ionization (ESI) mass spectrometry (Finnigan LCQ). The ESI mass spectra measured for untreated dAdpG and for dAdpG evaporated by LIAD are essentially identical. They are dominated by protonated dAdpG. No signals for free nucleobases were detected. Therefore, *LIAD is concluded to allow the evaporation of neutral dinucleoside phosphates into the gas phase without fragmentation.*

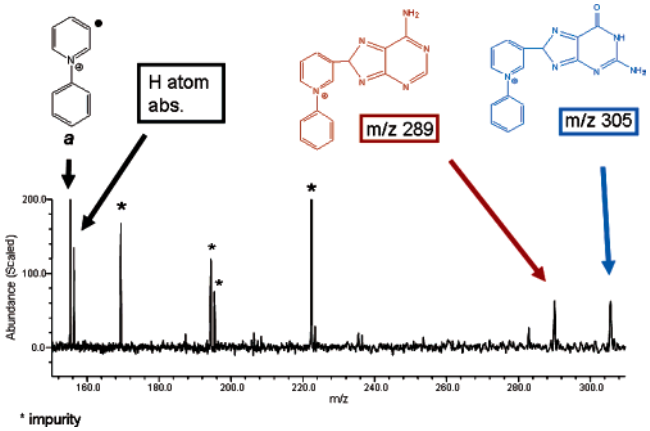


Figure 1. Reaction of the more electrophilic radical **a** with dAdpG. The stars indicate impurities arising, for example, from reactions of **a** with air.

The reactivity of the phenyl radicals **a** and **b** was first examined toward the nucleosides thymidine and guanosine (evaporated by LIAD) since these reactions have been studied in solution for the neutral phenyl radical. The neutral phenyl radical has been reported to react in solution with guanosine by H-atom abstraction and with thymidine by both H-atom abstraction and addition.[1a] The same reactions were observed for the radicals **a** and **b** in the gas-phase experiments, independent of the evaporation method employed (thermal heating or LIAD).[5c,e] These findings indicate that this experimental approach provides a *useful tool for the study of radical reactions of DNA components.*

Examination of the reactions of the charged *N*-phenyl-3-dehydropyridinium radical (**a**) with dAdpG (Figure 1) and with several other dinucleoside phosphates demonstrates that H-atom

† Current address: Department of Chemistry, University of California, Davis, CA.
‡ Current address: ExxonMobil, Fairfax, VA.
§ Current address: San Juan, Puerto Rico.

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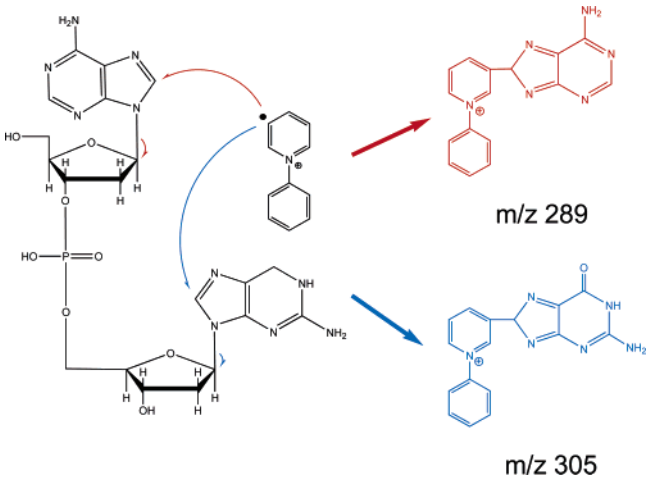
Scheme 2



abstraction (H-abs.) and addition to nucleobases occur readily. The addition is always followed by N−C bond cleavage and elimination of the rest of the molecule (Scheme 2). Our previous comparison of the reactivity of **a** toward sugars, nucleobases, and thymidine (evaporated thermally) suggests that H-abstraction occurs predominantly from the sugar moiety in nucleosides (although the thymine methyl group also acts as a H-donor).[5e] The high percentage of H-atom abstraction by **a** from dAdpG (Figure 1) and from other dinucleoside phosphates is consistent with the H-abstraction occurring predominantly at the sugar moiety in dinucleoside phosphates.

Comparison of the products obtained in the reactions of radical **a** with dApdG, dApdC, dCpT, TpT, dApdA, and dGpdG shows that the structure of the dinucleoside phosphate has a strong influence on these radical reactions. For example, while dApdG (H-abs. 44%, addition to G 25%, addition to A 31%), dApdA (H-abs. 88%, addition to A 12%), and dGpdG (H-abs. 69%, addition to G 31%) react with **a** by addition/elimination as well as H-atom transfer, dCpT and TpT only react by H-atom transfer. Examination of the reactions of dApdC (H-abs. 90%, addition to A 10%) demonstrates that the dissimilar behavior of the different dinucleoside phosphates arises from the fact that *the radical appears to add to purine bases but not to pyrimidine bases in these substrates*. This was a surprising finding because solution studies do not provide conclusive evidence for the ability of phenyl radicals to add to purine bases, although addition to pyrimidine bases was conclusively demonstrated.[1a] Further, our earlier gas-phase studies have revealed that radical **a** adds to isolated purine and pyrimidine bases and to thymine in thymidine.[5e] The reasons for the different behavior of the phenyl radical toward dinucleoside phosphates are not clear at this time.

The influence of the structure of the attacking radical on reactions with dinucleoside phosphates is obvious when comparing the reactions of radical **a** to those of the isomeric *N*-(3-dehydrophenyl)-pyridinium radical (**b**; Scheme 1). Radical **b** reacts with all the dinucleoside phosphates by predominant H-atom abstraction. No products corresponding to addition to a nucleobase were observed. The differences in the two radicals' behavior can be related to their dissimilar electrophilicities.[5d,e] Radical **a** is substantially more electrophilic than radical **b**, as reflected by the radicals' calculated vertical electron affinities (EA_v = 5.8 and 4.9 eV, respectively; B3LYP/6-31+G(d); EA is defined here as the energy released when an electron is added to the radical orbital at a frozen geometry). This difference has been demonstrated earlier to make **a** much more reactive than **b** toward simple organic substrates.[5d,e] Furthermore, studies on aromatic substrates have demonstrated[5e,f] that an increase in the EA of a radical facilitates both the addition and H-atom abstraction reactions, but more the former. This is exactly what was observed here for the dinucleoside phosphates: the less electrophilic radical **b** favors H-atom abstraction from a sugar moiety of dinucleoside phosphates, while the more electrophilic radical **a** also undergoes addition/elimination reactions with the purine bases. On the basis of these results, *an increase in a radical's electrophilicity may lead not only to more extensive damage to the dinucleoside phosphates but also damage to different parts of the substrate.*

In conclusion, we have demonstrated here that LIAD combined with mass spectrometry provides a powerful tool for the study of reactions of phenyl radicals with dinucleoside phosphates. Further, phenyl radicals are shown to be able to attack dinucleoside phosphates at both the sugar and base moieties. Purine bases were found to be more susceptible to radical attack than pyrimidine bases. The more electrophilic the radical, the more it favors addition to a purine base over H-atom abstraction from a sugar moiety. Therefore, the extent as well as the type of damage that a phenyl radical causes to a dinucleoside phosphate depends both on the radical's structure and the composition of the dinucleoside phosphate.

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Supporting Information Available: ESI mass spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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