Intrinsic electrophilic properties of nucleosides: Photoelectron spectroscopy of their parent anions

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The nucleoside parent anions 2′-deoxythymidine−, 2′-deoxycytidine−, 2′-deoxyadenosine−, uridine−, cytidine−, adenosine−, and guanosine− were generated in a novel source, employing a combination of infrared desorption, electron photoemission, and a gas jet expansion. Once mass selected, the anion photoelectron spectrum of each of these was recorded. In the three cases in which comparisons were possible, the vertical detachment energies and likely adiabatic electron affinities extracted from these spectra agreed well with the values calculated both by Richardson et al. [J. Am. Chem. Soc. 126, 4404 (2004)] and by Li et al. [Radiat. Res. 165, 721 (2006)]. Through the combination of our experimental results and their theoretical calculations, several implications emerge. (1) With the possible exception of dG−, the parent anions of nucleosides exist, and they are stable. (2) These nucleoside anions are valence anions, and in most cases the negative charge is closely associated with the nucleobase moiety. (3) The nucleoside parent anions we have generated and studied are the negative ions of canonical, neutral nucleosides, similar to those found in DNA. © 2007 American Institute of Physics. [DOI: 10.1063/1.2774985]

INTRODUCTION

It has long been known that ionizing radiation can cause genetic damage. However, the high energy particles and photons which constitute ionizing radiation are not themselves directly responsible for damaging DNA and thus causing mutations.1 Instead, the radicals and electrons formed by the interaction of ionizing radiation with matter (especially water) are the principal instigators of DNA and RNA damage.1,2 The radicals cause damage through chemical reactions. The electrons, on the other hand, can induce further ionization, producing even more radicals and electrons. Upon losing energy and thermalizing, some electrons also form highly reactive solvated electrons which themselves sit at the headwaters of further chemical reactions. Ultimately, however, the most prevalent species generated by ionizing radiation are secondary electrons, produced by cascades of energy-losing ionization and electron scattering events, and of these, most have energies below ~10 eV.

Nevertheless, despite their abundance, low energy electrons have not been considered to be important actors in radiation damage to DNA until relatively recently. That began to change with the seminal work of Sanche and co-workers3–5 who demonstrated in electron impact experiments on thin films of plasmid DNA that single strand breaks occur in DNA due to electrons with energies below ~4 eV and that double strand breaks occur at electron energies as low as ~10 eV. This is astonishing given that much of this damage occurs at energies significantly below the ionization threshold of DNA. The resonant character of the experimental evidence points to these processes occurring through the formation of transient anions on the subunits of DNA, quite likely on the nucleic acid bases themselves. While the mechanism by which this leads to strand breaks is still under debate, there may well be a coupling between temporary (transient) anions and their stable anions, whereby the former serve as stepping stones to the latter which in turn are involved in strand breaks.6–8 Thus, the anions of DNA’s subunits appear to play a significant role in mutagenesis.

Electron-nucleobase interactions have been studied extensively. In gas phase studies, temporary anions of nucleobases have been studied by electron transmission spectroscopy,9 Dissociative electron attachment resulting from the interaction of gaseous nucleobases and free electrons has been studied as a function of electron energy,10–12 and anion photoelectron spectroscopy has probed deprotonated base anions.13,14 Among parent anions of canonical nucleobases, most have been found by anion photoelectron spectroscopy15–17 and Rydberg electron transfer18–20 to be ground state, dipole bound states, although valence anions of canonical uracil21 and of rare tautomers of all five nucleobases22,23 have also been observed and studied. In the condensed phase, parent valence anions of nucleobases have been studied by electron spin resonance spectroscopy.24–26 In addition to experimental studies, theoretical work also abounds.27–33 In fact, theory had predicted the viability of dipole bound, nucleobase anions, and it was that prediction which motivated the early work on gas phase, intact (parent), nucleic acid base anions.34

Among electron interactions with nucleosides and nucleotides, dissociative electron attachment studies with both gaseous thymidine and uridine and with thin films of thymidine constitute the main experimental work reported.35–38 Theoretical work is quite prevalent, however, with much of it utilizing electron-nucleotide interactions to model proposed mechanisms by which low energy electrons induce single strand breaks.39–49 In the condensed phase, electron spin resonance studies have also been conducted on the radical anions of nucleosides and nucleotides.26,50
While anions of nucleosides and nucleotides can exist in condensed phase environments, there have been no reports to date of gas phase, parent valence anions of nucleosides or nucleotides in the experimental literature. This is not necessarily surprising when one considers that, except for the case of uracil, the parent valence anions of the canonical tautomers of the nucleic acid bases have not been seen in mass spectra, i.e., they have not been shown to be stable in isolation. Nevertheless, it seemed to us that both nucleosides and nucleotides should form stable, parent, valence anions in isolation. After all, if even marginal solvation can stabilize the valence anions of nucleobases [as it does in the cases of uracil−(Xe)1, uracil−(water)1, thymine−(water)1, cytosine−(water)1, and adenine−(water)1],16,17 then surely the sugar moieties within nucleosides and nucleotides should be able to stabilize them (in these cases through chemical bonding rather than solvation, however).

Here, we report the formation of gas phase, parent (intact), valence anions of several nucleosides, i.e., they do indeed exist as stable anions. These were generated in a novel source, identified by mass spectrometry and characterized by anion photoelectron spectroscopy. Our goal was to explore the intrinsic electrophilic properties of isolated, intact nucleoside molecules by attaching electrons to them and characterizing the resultant parent negative ions. This necessitated forming them under conditions where third body cooling collisions could carry away excess energy and stabilize them. This approach is in contrast to free electron attachment reducing the degree of undesired dissociative attachment processes as is done in electron-vapor dissociative attachment studies. There, the transient anion has little choice but to dissociate into neutral and anionic fragments or to autodetach its electron. Since dissociative attachment processes are prevalent in radiobiology, this is an important method for probing DNA damage pathways. We see our studies of parent nucleoside anions as complementary to the dissociative attachment work with nucleosides.

**EXPERIMENT**

One should not overlook the importance of sources for forming anions of biomolecules. In particular, preparing parent anions of most biomolecules is a vexing problem. If one could simply vaporize the biomolecule of interest and expand it in a cooling jet of inert gas while at the same time injecting electrons into the mix (through any of several methods), then preparing parent anions of biomolecules which have positive adiabatic electron affinities would be a relatively standard experimental procedure. However, since most biomolecules of significant size tend to be involatile and easily decompose upon heating, the initial thermal evaporation step described above is of limited utility for bringing them into the gas phase. Even in cases in which the evaporation of intact biomolecules is partly successful, these are often accompanied by undesired decomposition products. Furthermore, modern methods such as electrospray and conventional matrix assisted laser desorption ionization (MALDI) do not solve this problem. Parent anion formation is relatively rare via electrospray sources. Negative ions generated by electrospray tend to have lost a hydrogen atom, i.e., they are deprotonated neutral species. Furthermore, many of them are also multiply negatively charged. While these are very important species in biological systems, they are not the parent anionic species that we seek to study here. Conventional MALDI often also has many of the same problems, leading to anion products which can be viewed as anionic fragments of parent anions. In short, there were no reliable methods available for forming parent anions of most biomolecules.

To solve this problem, we have developed a novel source for forming parent anions of involatile molecules. The idea was to bring bursts of (1) gaseous neutral biomolecules, (2) low energy electrons, and (3) rapidly expanding inert gas atoms together at the right time and in the right place. To accomplish the first task, we utilized pulsed laser, infrared desorption. The work of de Vries et al.51 provided the most direct guidance for implementing infrared desorption of biomolecules. The second task was accomplished using pulsed laser, photoelectron emission. There, we relied on the work of Boesl et al.52 for guidance. Both of these techniques had been pioneered by Schlag et al.53 The third task of supplying a collisionally cooling jet of helium was accomplished in a routine fashion, using a pulsed gas valve.

The geometric arrangement of this hybrid source is shown in Figs. 1(a) and 1(b). Figure 1(a) shows the source with a metal wire photoemitter, while Fig. 1(b) shows it with an yttria disk photoemitter. Infrared desorption was accomplished by directing an attenuated power beam of 1064 nm light (first harmonic frequency) from a pulsed Nd:YAG (yttrium aluminum garnet) laser onto a slowly moving, graphite bar which was thinly coated with the sample. Biomolecules were desorbed from the bar due to the absorption of infrared photons by the graphite and its ensuing ultrafast temperature rise. Coordinated with the IR pulses were pulses from a second Nd:YAG laser operated at its second harmonic frequency. In an earlier version of this source [Fig. 1(a)], we used metals having specific work functions as photoemitters.54 In the present improved version, however, we often use yttrium oxide as the photoemitter. Its work function of ~2 eV is just below the photon energy of the second harmonic frequency of a Nd:YAG laser (2.33 eV), and this leads to the photoemission of rather low energy electrons, their attachment reducing the degree of undesired fragmentation. We learned of yttria as a photoemitter of electrons from the work of Nakajima et al.55 Thus, by coordinat-
conserving relationship, $h\nu = \text{EBE} + \text{EKE}$, where $h\nu$ is the photon energy, EBE is the electron binding energy, and EKE is the electron kinetic energy. Knowing the photon energy and measuring the electron kinetic energy, one determines the electron binding energies of the observed transitions.

RESULTS

Figure 2 shows the mass spectrum of the parent anion of cytidine. It is one of seven nucleoside parent anions generated by the source described in the text and shown schematically in Fig. 1.

For purine-based nucleoside anions, the rising photoelectron intensities at EBE $> 2.3$ eV are due to photodetachment transitions from the ground vibrionic state of the nucleoside anion to the first excited vibrionic state of its neutral counterpart. In the cases of the four pyrimidine-based nucleoside anions, however, the rising intensities at EBE $> 3.0$ eV may be due to ground state anion to first excited state neutral transitions, but they may also include contributions from very low kinetic energy photoelectrons (low EKE) which are difficult to fully eliminate from that region of the spectra.

In this paper, we will focus on the spectral bands in the EBE window between $0.2$ and $2.3$ eV. The EBE values at the maximal photoelectron intensities of these bands correspond to the optimal Franck-Condon overlaps of vibrational wave functions during photodetachment transitions between ground state anions and the ground states of their corresponding neutrals. This energetic quantity is the vertical detachment energy (VDE). The energy difference between the lowest vibrational level of the ground electronic state of the anion and the lowest vibrational level of the ground electronic state of its corresponding neutral is the adiabatic electron affinity (EA$_a$). While the VDE has a defined value in each spectrum, extracting a high-confidence EA$_a$ value from a photoelectron spectrum requires the presence of resolved vibrational structure which is not exhibited in these bands. Nevertheless, some information about the EA$_a$ value is available. When only the lowest vibrational level of the anion is populated and there is Franck-Condon overlap between the anion and its neutral, then the EBE value at the photoelectron intensity threshold is equal to the EA$_a$ value. Since hot bands are not uncommon, however, the EA$_a$ value often lies between the threshold and the VDE, but in any case, EA$_a$ $\approx$ VDE always holds for stable anions. Thus, one can usually judge whether a theoretically predicted EA$_a$ value is consistent with a measured anion photoelectron spectrum. Measured VDE values and estimated EA$_a$ values for the nucleoside systems we have studied here are presented in Table I. These are the first experimental determinations of these quantities for nucleosides and their anions.
The electron binding energy (EBE) is a measure of the energy required to remove an electron from a molecule. In this case, the EBE values for the nucleoside anions are lower than the VDE values of their corresponding neutral counterparts. This suggests that the anionic nucleosides are more stable than their neutral counterparts.

The photoelectron spectra of the nucleoside anions show close resemblance between the photoelectron spectra of cytidine$^-$ and deoxycytidine$^-$, as well as between dC$^-$ and dC$^-$. This is due to the similar structures of these molecules. However, there are also differences, as indicated by the VDE and EA values.

The DISCUSSION section explains that while the nucleosides' neutral configurations were limited to those that exist in natural DNA, allowing structures of their anions to be optimized, the calculations or theory may be due to the diminished Franck-Condon overlap between the lowest vibrational levels of dA$^-$ and dA. In fact, the similarly small EA value from the calculation of Sevilla et al. suggests that this latter explanation may be the better one, although contrary to the negative value of EA computed by that calculation, our ability to prepare and study dA$^-$ in beams suggests that it is a stable anion.
dA− is a reflection of the differences between pyrimidine-based nucleosides and purine-based nucleosides and theory’s ability to account for them. On the whole, however, the agreement between theory and experiment must be seen as being excellent.

Furthermore, while theoretical values for EAa and VDE were calculated for dG and dG− by Schaefer et al., we were not able to record the spectrum of dG−. We did, however, measure the photoelectron spectrum of the ribonucleoside anion, guanosine−. If the shift described above between deoxy- and ribonucleoside anion spectra holds, then the spectrum of dG− should look like that of guanosine−, just located at slightly lower EBE values. If so, it would not look like a diffuse dipole bound anion, the photoelectron spectra of which display distinctive signatures, characterized by a single narrow peak at very low EBE. Nevertheless, the shape of the guanosine− spectrum is different from the others, de−viating considerably from those of its cousins, dA− and adenosine−, wherein the lower EBE band of the guanosine− spectrum is indistinct, even though the shape of its high EBE feature maintains the purine-based nucleoside anion family trait. It seems plausible that the deviation of its spectral shape may be somehow related to the diffuse excess electron character predicted by both theoretical studies.

Through the combination of our experimental results on nucleoside anions and the theoretical calculations of Schaefer et al. and of Sevilla et al., several implications emerge. (1) With the possible exception of dG−, the parent anions of nucleosides exist, and they are stable. (2) The electron affinities of the nucleosides are greater than those of their corresponding nucleobases. (3) The nucleoside parent anions we have generated and studied by photoelectron spectroscopy are the negative ions of canonical, neutral nucleosides, similar to those found in DNA and perhaps to those in some RNA configurations.

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