

Photoelectron spectroscopic studies of 5-halouracil anions

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The parent negative ions of 5-chlorouracil, UCl^- and 5-fluorouracil, UF^- have been studied using anion photoelectron spectroscopy in order to investigate the electrophilic properties of their corresponding neutral halouracils. The vertical detachment energies (VDE) of these anions and the adiabatic electron affinities (EA) of their neutral molecular counterparts are reported. These results are in good agreement with the results of previously published theoretical calculations. The VDE values for both UCl^- and UF^- and the EA values for their neutral molecular counterparts are much greater than the corresponding values for both anionic and neutral forms of canonical uracil and thymine. These results are consistent with the observation that DNA is more sensitive to radiation damage when thymine is replaced by halouracil. While we also attempted to prepare the parent anion of 5-bromouracil, UBr^- , we did not observe it, the mass spectrum exhibiting only Br^- fragments, i.e., 5-bromouracil apparently underwent dissociative electron attachment. This observation is consistent with a previous assessment, suggesting that 5-bromouracil is the best radio-sensitizer among these three halo-nucleobases. © 2011 American Institute of Physics. [doi:10.1063/1.3525623]

I. INTRODUCTION

Upon exposure of biological tissue to ionizing radiation, the main agents of genetic damage are low energy electrons, holes and other free radicals. While free radical induced damage has been studied extensively in the past, it has recently been shown that low energy electrons also make significant contributions to radiation induced damage, viz., they initiate reactions which ultimately lead to single and double strand breaks in DNA.¹⁻³ Electron attachment to nucleic acid base moieties in DNA is believed to be an important part of a complex mechanism involved in radiation damage, and this has motivated several studies involving electron interactions with nucleobases.

Substitution of thymine by 5-halouracils in cellular DNA results in enhanced sensitivity to ionizing and ultraviolet radiation and an increase in cell death.⁴⁻⁹ The radio-sensitization properties of 5-halouracils are ultimately governed by their tendency to undergo dissociative electron attachment. In this process, electron attachment initially results in the formation of short-lived 5-halouracil anions, which then dissociate into very reactive uracil-5-yl radicals and halide anions, as has been demonstrated in both gas-phase¹⁰ and computational studies.¹¹ Even so, direct electron attachment to halouracils in DNA is probably not the main process by which transient halouracil anions are initially formed there. The more likely path is believed to be electron transfer from normal DNA bases, since these are far more abundant in the strand and also are thought to have lower electron affinities.¹² For this reason, it is important to compare the adiabatic electron affinity values of regular nucleobases (such as uracil and especially thymine)

with those of halouracils, since this difference impacts the ability of halouracils to scavenge electrons from regular nucleobases to form transient halouracil anions which can then undergo dissociative electron attachment.

The majority of experimental¹³⁻¹⁵ and theoretical¹⁶⁻²³ studies on canonical nucleic acid bases suggest that, in isolation, they support dipole bound anions, and for all practical purposes, do not form stable valence anions. For such dipole bound states, both their adiabatic electron affinities (EA) and their vertical detachment energies (VDE) values are very small (~ 90 meV) and essentially identical. Some theoretical studies suggest that valence anions of uracil and thymine are marginally stable, implying that their EA values are slightly positive, but even these values are predicted to be near zero.²⁴⁻²⁶ However, valence anions of nucleobases are observed experimentally when sufficiently stabilized with solvent molecules.^{15,27-30} Noncanonical valence anions of rare tautomers of nucleic acid bases have also been observed experimentally, but these are not relevant to the present topic.³¹

While there have been no experimental measurements of EA and VDE values for 5-halouracils, several relevant studies have been conducted. Experimentally, Scheer and co-workers have studied 5-halouracils using electron transmission spectroscopy.³² Also, low-energy electron interaction with 5-halouracils has been studied in aqueous solution.³³⁻³⁵ Theoretically, Wetmore *et al.*³⁶ and Li *et al.*¹¹ have both calculated electron affinity values for halouracils, finding their values to be larger than the electron affinities of both uracil and thymine molecules.^{24,25} Also, the EA value of the uracil-5-yl radical is predicted to be relatively high (2.34 eV), consistent with its expected reactivity.¹¹ In addition, Sommerfeld has studied anionic states of 5-chlorouracil and proposed a mechanism for dissociative electron attachment.³⁷

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Furthermore, the pairing of 5-halouracils with adenine is predicted to result in complexes which have lower EA values than the individual 5-halouracils, suggesting that their ability for electron capture in double stranded DNA may be reduced relative to single stranded DNA.^{38,39}

In addition, the halo-nucleosides, 5-bromodeoxyuridine and 5-iododeoxyuridine may also have potential as radiosensitizers.^{40,41} Time-resolved, femtosecond laser spectroscopy measurements on aqueous solutions of halouridines showed that ultrafast transfer of pre-solvated electrons to halouridines is responsible for radical formation through dissociative attachment.^{34,35} In fact, some halouridines have already undergone clinical trials for the treatment of cancer.⁴²

In the present work, we report the experimentally measured vertical detachment energy values of 5-chlorouracil and 5-fluorouracil anions together with estimates of the adiabatic electron affinity values of their corresponding neutral molecules. We then compare these values not only with those predicted by theoretical studies, but also with those of the natural nucleobases, uracil, and thymine.

II. EXPERIMENTAL

Negative ion photoelectron spectroscopy is conducted by crossing a mass-selected beams of negative ions with a fixed-frequency beam of photons and energy-analyzing the resultant photodetached electrons. This technique is based on the energy conserving relationship, $h\nu = \text{EKE} + \text{EBE}$, where EKE is electron kinetic energy, EBE is electron binding energy, and $h\nu$ is the photon energy. In the experiments reported here, negative ions were formed in a nozzle-ion source. There, the 5-halouracil of interest was placed in a stagnation chamber, heated to 160–180 °C, and co-expanded with 2 atm of argon gas through a 23 μm nozzle. A hot, negatively biased filament injected low energy electrons, in the presence of a weak magnetic field, into the resulting gas expansion, producing negative ions. These were then extracted, collimated, and transferred into the flight tube of a 90° magnetic sector mass spectrometer with a typical mass resolution of 400. The mass-selected anions of interest were then crossed with the intracavity laser beam of an argon ion laser, operating at 2.54 eV/photon, and the resulting photodetached electrons then energy analyzed in a hemispherical electron energy analyzer with a typical resolution of 25 meV. Photoelectron spectra of 5-halouracils anions were calibrated against the well-known spectrum of O^- . A detailed description of our apparatus has been given elsewhere.⁴³

III. RESULTS AND DISCUSSION

Our measured photoelectron spectra of the 5-chlorouracil negative ion, UCI^- and of the 5-fluorouracil negative ion, UF^- , recorded with 2.54 eV photons, are presented in Fig. 1. Both spectra are characterized by single, broad bands, with measured VDE values (the VDE is the EBE value corresponding to the maximum in the spectral intensity profile) of 1.01 and 0.88 eV, respectively. These values are in good agreement with the theoretical results of Li *et al.*,

TABLE I. The values of VDE and EA for UCI and UF extracted from our anion photoelectron spectra and compared to theoretically calculated values. All energies in eV.

UX	VDE (expt)	VDE (theor) ^a	EA (expt)	EA (theor) ^a	EA (theor) ^b
UCI	1.01	1.20	0.45–0.65	0.60	0.58
UF	0.88	1.14	0.30–0.50	0.48	0.45

^aRefers to Ref. 11.

^bRefers to Ref. 36.

whose calculated VDE values are 1.20 eV for UCI^- and 1.14 eV for UF^- .¹¹ The same study also reported calculated EA values of 0.60 eV for UCI and 0.48 eV for UF, values which are very similar to those calculated by Wetmore *et al.*, i.e., 0.58 and 0.45 eV, respectively.³⁶ These EA values are consistent with those estimated from our spectra. An EA value can often be estimated from the low EBE side of the spectral band, after accounting for likely vibrational hot band contributions. This puts the EA value of UCI in the range of 0.45–0.65 eV and EA value of UF in the range of 0.30–0.50 eV. The experimentally determined values of VDE and EA for both systems are presented in Table I, together with theoretically predicted values for comparison. In addition to their agreement with theory, our experimentally

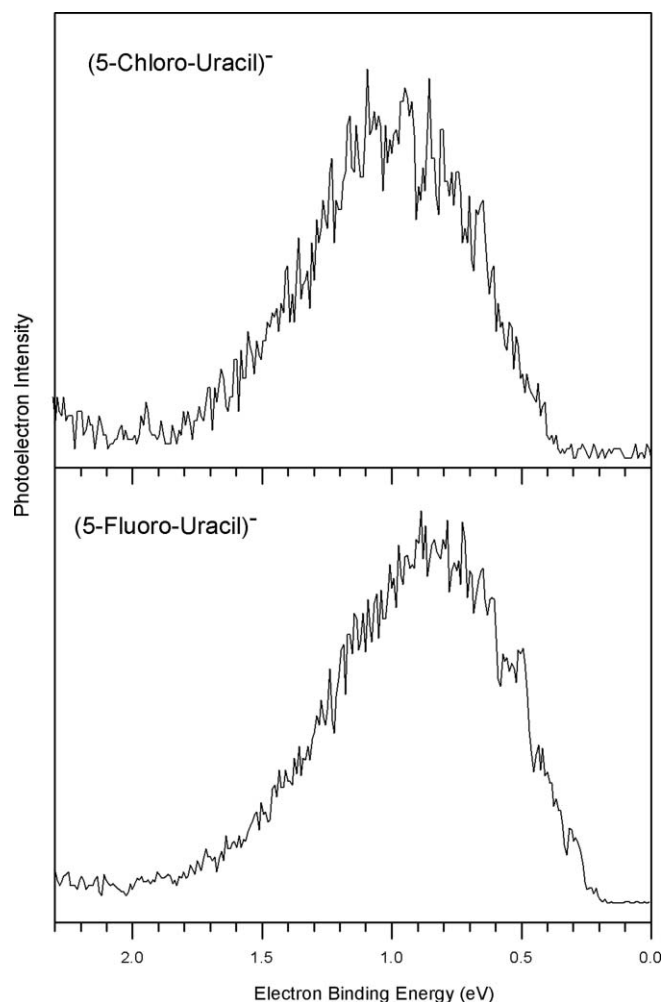


FIG. 1. Negative ion photoelectron spectra of the 5-chlorouracil and 5-fluorouracil anions, both recorded with 2.54 eV photons.

determined values of VDE and EA for UCI^- and UCI and for UF^- and UF , respectively, also show them to be significantly larger than the same quantities for the uracil and thymine cases, helping to explain why the incorporation of halouracils into DNA results in greater sensitivity to radiation damage.

While we observed the parent negative ions of 5-chlorouracil, UCI^- , and 5-fluorouracil, UF^- , we did not observe the parent negative ion of 5-bromouracil, UBr^- . Two theoretical studies predict an EA value for UBr^- , i.e., 0.51 eV (Ref. 11) and 0.64 eV (Ref. 36), which is comparable to that of UCI and larger than that of UF , and both find that UBr^- dissociates more readily than the anions, UCI^- and UF^- . Furthermore, nanosecond pulse radiolysis experiments in aqueous solution found the lifetime of UBr^- to be 7 ns, much shorter than the lifetimes of UCI^- (4.9 μs) or UF^- (>15 μs).³³ Even though there is a gas phase study⁴⁴ that reports a UBr^- lifetime of $\sim 50 \mu\text{s}$, “gas phase” theoretical results, aqueous phase experimental EPR results at low temperatures⁴⁵ and pulse radiolysis studies at room temperature⁴⁶ studies all agree that UBr^- dissociates more readily than either UCI^- or UF^- . Thus, on balance, it is not surprising that we did not observe the parent anion of 5-bromouracil, UBr^- , on our detection time scale of $\sim 10 \mu\text{s}$. The fact that we saw the parent anions, UCI^- and UF^- , but that we did not see the parent anion, UBr^- in our mass spectra is in agreement with the observation that, when incorporated into DNA, bromouracil is the best radio-sensitizer among the halouracils studied here. While we did not study iodouracil in this study, one might presume that it is probably an even better radio-sensitizer than bromouracil.

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