

Dipole bound, nucleic acid base anions studied via negative ion photoelectron spectroscopy

J. H. Hendricks, S. A. Lyapustina, H. L. de Clercq, J. T. Snodgrass,^{a)} and K. H. Bowen^{b)}
Department of Chemistry, Johns Hopkins University, Baltimore, Maryland 21218

(Received 4 December 1995; accepted 14 March 1996)

The anions of the nucleic acid bases, uracil and thymine, were studied by negative ion photoelectron spectroscopy. Both monomer anions exhibit spectroscopic signatures that are indicative of dipole bound excess electrons. The adiabatic electron affinities of these molecules were found to be 93 ± 7 meV for uracil and 69 ± 7 meV for thymine. No conventional (valence) anions of these molecules were observed. © 1996 American Institute of Physics. [S0021-9606(96)03519-2]

Nucleic acid bases govern the storage and transfer of genetic information. When ionizing radiation interacts with living organisms, one of the mechanisms for genetic damage involves the attachment of electrons to DNA and RNA bases. The resultant radical anions then participate in a chain of chemical reactions that can lead to permanent alteration of the original bases and to genetic damage. Nucleic acid base anions thus play an important role in radiation-induced mutagenesis. Because of these considerations, nucleic acid base radical anions have been the subject of numerous experimental studies in the condensed phase.¹ By contrast, gas-phase experimental work on such anions has been limited to three reports: an early mass spectral observation² of an uncharacterized form of the thymine molecular anion, an electron transmission spectroscopic study^{3,4} of uracil and its temporary anions, and very recently, Rydberg electron transfer studies⁵ of nucleic acid base dimer anions. Until now, however, most information about the nature and extent of excess electron binding in isolated (gas-phase) nucleic acid base molecular anions has come from theoretical studies.

There have been several calculations on isolated uracil and thymine anions, specifically those by Pullman,⁶ Compton,³ Sevilla,^{7,8} and Adamowicz.^{9,10} While none of these found stable conventional (valence) anions directly, Sevilla's scaling of his theoretical adiabatic electron affinity (EA_a) values to experimentally known EA_a values of related molecules implied the existence of stable, isolated covalently bound anions for both uracil and thymine. In this way, Sevilla predicted adiabatic EA_a values of +0.4 and +0.3 eV for uracil and thymine, respectively.⁸ By contrast, Adamowicz's calculations found stable, albeit fragile, nucleic acid base anions of uracil⁹ and thymine¹⁰ in which their excess electrons were bound by the dipolar fields of the bases.

The nucleic acid bases, uracil and thymine, possess large dipole moments, i.e., ~ 5 D.⁹⁻¹¹ Due to extensive theoretical¹² as well as experimental¹³ work, it is well established that excess electrons can be bound by the dipole fields of neutral molecular or cluster systems to form anions (often loosely referred to as "dipole bound anions"), if the dipole

moment of the system is larger than ~ 2.5 D.¹⁴ The nucleic acid base anions found in Adamowicz's calculations exhibit the properties expected of negative ions having dipole bound excess electrons. Their excess electron clouds were extremely diffuse and anisotropic; their excess electron binding energies were very small, i.e., with predicted EA_a 's of 86 meV and 88 meV for uracil and thymine, respectively; and their structures were virtually the same as those of their corresponding neutrals.

Here, we present experimental results pertaining to the nature of isolated, gas-phase nucleic acid base anions. In this study, the monomer anions of the nucleic acid bases, uracil and thymine (5-methyluracil), were generated using a supersonic expansion nozzle ion source and characterized via negative ion photoelectron spectroscopy. In a companion paper by Desfrancois, Abdoul-Carime, and Schermann, gas-phase nucleic acid base monomer anions were generated through electron transfer collisions between the bases and laser-excited Rydberg atoms and characterized via the dependence of anion formation rates on Rydberg electron quantum numbers (energies). These two experiments provide different, yet rather complementary approaches to the study of these species, both in terms of anion formation mechanisms and in the manner of their characterization, and in most respects, they are in good agreement.

Negative ion photoelectron spectroscopy is conducted by crossing a mass-selected beam of negative ions with a fixed-frequency photon beam and energy analyzing the resultant photodetached electrons. This is a direct approach to the determination of electron binding energies (EBE), relying as it does on the relationship, $h\nu = \text{EBE} + \text{EKE}$, in which $h\nu$ is the photon energy and EKE is the measured electron kinetic energy. Our apparatus has been described in detail previously.¹⁵ The spectra were calibrated against the well-known photodetachment spectra¹⁶ of O^- and NO^- , the resolution (FWHM) of our electron analyzer was ~ 30 meV, and photodetachment was accomplished with 220 circulating Watts of 2.540 eV photons. In a supersonic expansion ion source, relatively low energy electrons are injected directly into the high density portion of an expanding jet in the presence of weak axial magnetic fields, and negative ions are extracted from the resulting microplasma.¹⁷ The negative ion formation environment involves the attachment of even

^{a)}Taylor Technology, Inc., Princeton, NJ 08540.

^{b)}Author to whom correspondence should be addressed.

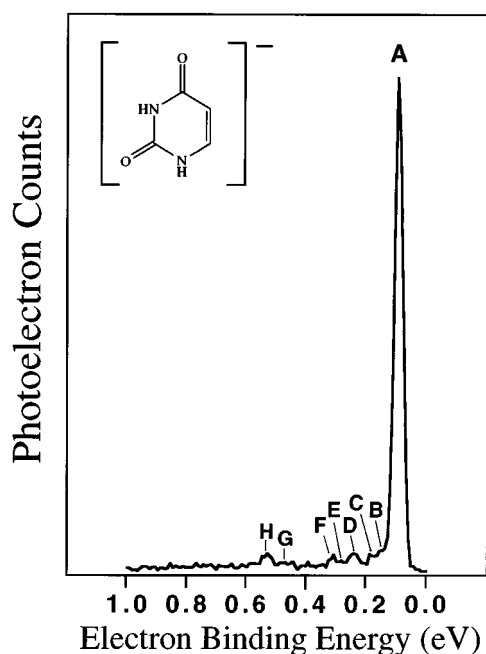


FIG. 1. The photoelectron spectrum of the uracil molecular anion, (U)⁻, recorded with 2.540 eV photons.

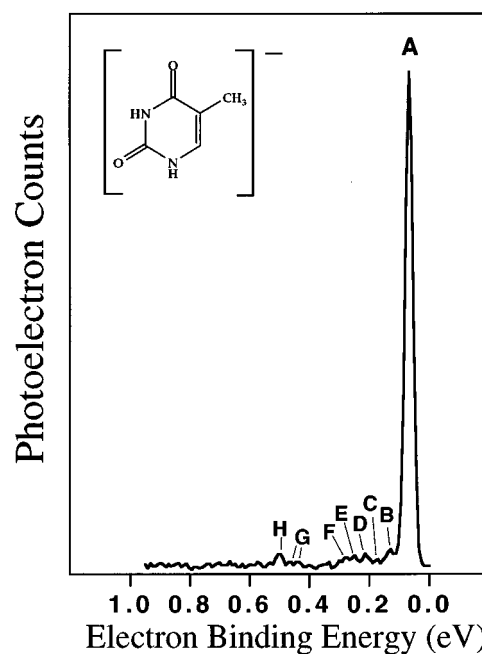


FIG. 2. The photoelectron spectrum of the thymine molecular anion, (T)⁻, recorded with 2.540 eV photons.

lower energy secondary electrons to target species in the presence of many cooling collisions. A wide range of anion formation conditions are possible with this source, and the fact that it has generated so many different, fragile dipole bound anions¹⁸ attests to its ability to provide very gentle anion formation conditions as part of its repertoire. To form the expansion jet in the present experiments, samples of uracil and thymine powder were slowly heated to 160–170 °C inside the stagnation chamber of this source and co-expanded with 3–4 atm argon gas through a 20 μm orifice. Just outside the nozzle, the resulting jet was typically subjected to 3–6 mA of ~40 eV primary electrons.

The photoelectron spectra of uracil and thymine anions are presented in Figs. 1 and 2, respectively. Each spectrum is characterized by a single, strong, sharp feature (peak A) at very low electron binding energies and by several much lower intensity features (B–H) at slightly higher electron binding energies. This is the distinctive photoelectron spectral signature of dipole bound anions. We have seen this spectral fingerprint in all of the many ground state, dipole bound anions that we have studied thus far,¹⁸ and it is unlike that of any other anionic species we have encountered. Essentially, the dominant peak (peak A) in each of these spectra is the origin “transition” between the ground states of the anion and its corresponding neutral. The fact that its electron binding energy is so small indicates that the excess electron is bound very weakly, while the relative strength and unusual narrowness of this peak (essentially instrumentally limited) implies that the structures of the anion and its corresponding neutral, in both cases, are very similar, if not identical. The weak peaks, B–H, in Figs. 1 and 2 are assignable as molecular vibrations¹⁹ of neutral uracil and thymine, respectively. Under the usual Franck–Condon analysis of

photoelectron spectra, this would imply a very slight structural difference between the anion and its neutral. Recent work by Johnson and co-workers,²⁰ however, casts doubt on the validity of conventional Franck–Condon treatments of vibrational features in photoelectron spectra of dipole bound anions, suggesting instead that vibronic coupling effects are substantially responsible for the observed vibrational features in these cases.

The vertical detachment energy (VDE) is the electron binding energy corresponding to the maximum in the origin peak (peak A) in these spectra. From the photoelectron spectra of uracil and thymine anions, their VDE's were determined to be 93 ± 7 meV and 69 ± 7 meV, respectively. The evidence strongly indicates that the anions of uracil and thymine have essentially the same structures as do their neutrals. Under these circumstances, the VDE is equal to the adiabatic electron affinity (EA_a), and hence the EA_a of uracil is 93 ± 7 meV, and the EA_a of thymine is 69 ± 7 meV. These values are in close agreement with theoretical predictions by Adamowicz and Oyler^{9,10} ($EA_a=86$ meV for uracil and $EA_a=88$ meV for thymine). Our objective in performing these experiments was to explore the nature of isolated, gas-phase nucleic acid base anions. Thus far, we have found clear evidence for dipole bound anions (ground states) of uracil and thymine, largely confirming both the qualitative and the quantitative predictions of Adamowicz's calculations. In the accompanying paper, Desfrancois and co-workers used the Rydberg electron transfer technique to recover electron affinity values ($EA_a=54$ meV for uracil and $EA_a=68$ meV for thymine) which are in accord with both Adamowicz's predictions and our results.

The issue of the stability of isolated, conventional (valence) anions of uracil and thymine is an intriguing one

which deserves comment. At this point, there are conflicting indications as to their existence as stable, gas-phase covalent molecular anions. Below, we review the evidence, beginning with that which tends to counsel against the stability of the isolated valence forms. (1) Of the several theoretical calculations^{3,6-10} conducted on anions of uracil and thymine, none found direct, quantitative evidence for stable valence anions. (2) When standard methods for forming (valence) anions of biological molecules (electrospray, atmospheric pressure chemical ionization, and fast atom bombardment) are utilized in mass spectrometry,²¹ the dissociative attachment (hydrogen loss) fragment anions, $(U-H)^-$ and $(T-H)^-$, are observed, but their parent anions, $(U)^-$ and $(T)^-$, are not. (3) Uracil has been investigated by electron transmission spectroscopy, and the results imply that its conventional anion is unstable.^{3,4} (4) We saw no spectroscopic evidence for conventional anions of uracil or thymine during this study, even though we made diligent searches over a wide range of source conditions. Given the photon energy used, we were implicitly looking for species²² with EA_a 's between 0–~2.5 eV, recalling that the EA_a 's of the species in question have been predicted to be 0.3–0.4 eV.⁸ Normally, a supersonic expansion ion source, with its many collisions, tends to make the most stable form of a given anion. An example is the nitromethane anion, a case in which even though both dipole bound and conventional anion forms are known to exist, the supersonic expansion ion source makes only the conventional anion, i.e., the more stable one.²³

Nevertheless, there is also evidence for the existence of stable, isolated conventional anions of uracil and thymine. (1) From theory, there are Sevilla's scaled calculations⁸ which imply the stability of isolated, conventional molecular anions of uracil and thymine, although not of adenine. (2) From mass spectrometry, there is a report of free $(T)^-$ being formed by a technique referred to as field-induced negative ion formation.² While one could count this observation as evidence for the existence of an isolated conventional anion of thymine, the observed anion was uncharacterized and might have been the dipole bound form. (3) When results from cyclic voltammetry measurements in solution were scaled to electron affinities of gas-phase calibrants, positive absolute electron affinities for uracil, thymine, and adenine were found (+0.80 eV, +0.79 eV, and +0.95 eV, respectively), implying the stability of their conventional anions in the gas phase.²⁴ (4) Desfrancois and co-workers have interpreted their Rydberg electron transfer results on uracil and thymine anions in terms of both dipole bound and conventional anions (see companion paper). In addition to a resonancelike peak in the spectra of both of these anions (clearly due to their dipole bound forms), these investigators also observed relatively flat, but nonzero ion intensity at lower Rydberg electron energies, and it is the occurrence of this nonzero baseline in their spectra (along with unusual broadening) that is the basis of the conventional anion portion of their interpretation. Their conclusions, as to the existence of isolated conventional anions of uracil and thymine, are given support by the fact that non-zero baselines were seen in their spectra for uracil and thymine anions, but not in their spec-

trum of the adenine anion, in accord with Sevilla's predictions.

While the stability of gas phase, conventional molecular anions of uracil and thymine may presently be an unresolved issue, ESR studies²⁵ leave little doubt that, in condensed media, the anions of uracil and thymine exist as stable, conventional covalent species. This raises interesting questions having to do with environment effects on the stability of dipole bound vs valence forms of these anions. In the condensed phase, solvation and multibody interactions surely play important roles in stabilizing the valence form of these anions.

This study was inspired by the work of L. Adamowicz,^{9,10} C. Desfrancois, and J. P. Schermann.⁵ We thank them as well as the following other colleagues for helpful discussions: J. Cadet, R. N. Compton, R. J. Cotter, D. E. Draper, C. C. Fenselau, L. Grossman, K. D. Jordan, M. D. Sevilla, C. A. Townsend, and T. D. Tullius. We also thank the National Science Foundation for its support of this work under Grant No. CHE-9007445.

¹M. D. Sevilla and D. Becker, Royal Society of Chemistry Special Review on Electron Spin Resonance, Chap. 5, Vol. 14 (1994), and references therein; C. von Sonntag, in *Physical and Chemical Mechanisms in Molecular Radiation Biology*, edited by W. E. Glass and M. N. Varma (Plenum, New York, 1991), and references therein; S. Steenken, *Chem. Rev.* **89**, 503 (1989).

²M. Anbar and G. A. St. John, *Science* **190**, 781 (1975).

³R. N. Compton, Y. Yoshioka, and K. D. Jordan, *Theor. Chim. Acta* **54**, 259 (1980); J. M. Younkin, L. J. Smith, and R. N. Compton, *ibid.* **41**, 157 (1976).

⁴K. D. Jordan and P. D. Burrow (private communications).

⁵C. Desfrancois, H. Abdoul-Carime, C. P. Schulz, and J. P. Schermann, *Science* **269**, 1707 (1995).

⁶B. Pullman and A. Pullman, *Rev. Mod. Phys.* **32**, 428 (1960); H. Berthod, C. Giessner-Prettre, and A. Pullman, *Theor. Chim. Acta* **5**, 53 (1966).

⁷A.-O. Colson, B. Besler, D. M. Close, and M. D. Sevilla, *J. Phys. Chem.* **96**, 661 (1992).

⁸M. D. Sevilla, B. Besler, and A.-O. Colson, *J. Phys. Chem.* **99**, 1060 (1995).

⁹L. Adamowicz, *J. Phys. Chem.* **97**, 11122 (1993).

¹⁰N. A. Oyler and L. Adamowicz, *Chem. Phys. Lett.* **219**, 223 (1994).

¹¹I. Kulakowska, M. Geller, B. Lesyng, and K. L. Wierzchowski, *Biochim. Biophys. Acta* **361**, 119 (1974); P. Mauret and J. P. Fayet, *C. R. Acad. Sci. Paris Ser. C* **267**, 2081 (1967); P. G. Jasien and G. Fitzgerald, *J. Chem. Phys.* **93**, 2554 (1990); R. D. Brown, P. D. Godfrey, D. McNaughton, and A. P. Pierlot, *J. Am. Chem. Soc.* **110**, 2329 (1988).

¹²W. R. Garrett, *Chem. Phys. Lett.* **5**, 393 (1970); *Phys. Rev.* **3A**, 961 (1971); O. H. Crawford and W. R. Garrett, *J. Chem. Phys.* **66**, 4968 (1977); K. D. Jordan and J. J. Wendoloski, *Chem. Phys.* **21**, 145 (1977); D. M. Chipman, *J. Phys. Chem.* **83**, 1657 (1979); A. Wallqvist, D. Thirumalai, and B. J. Berne, *J. Chem. Phys.* **85**, 1583 (1986); U. Landman, R. N. Barnett, C. L. Cleveland, D. Scharf, and J. Jortner, *J. Phys. Chem.* **91**, 4890 (1987); J. Simons and K. D. Jordan, *Chem. Rev.* **87**, 535 (1987).

¹³R. N. Compton, P. W. Reinhardt, and C. D. Cooper, *J. Chem. Phys.* **68**, 4360 (1978); R. D. Mead, K. R. Lykke, W. C. Lineberger, J. Marks, and J. I. Brauman, *ibid.* **81**, 4883 (1984); H. Haberland, C. Ludewigt, H.-G. Schindler, and D. R. Worsnop, *Phys. Rev. A* **36**, 967 (1987); K. H. Bowen and J. G. Eaton, in *The Structure of Small Molecules and Ions*, edited by R. Naaman and Z. Vager (Plenum, New York, 1988); S. T. Arnold, J. G. Eaton, D. Patel-Misra, H. W. Sarkas, and K. H. Bowen, in *Ion and Cluster Ion Spectroscopy and Structure*, edited by J. P. Maier (Elsevier, Amsterdam, 1989); R. Hashemi and E. Illenberger, *J. Phys. Chem.* **95**, 6402 (1991); C. Desfrancois, B. Baillon, and J. P. Schermann, S. T. Arnold, J. H. Hendricks, and K. H. Bowen, *Phys. Rev. Lett.* **72**, 48 (1994); C. Desfrancois, H. Abdoul-Carime, N. Khelifa, and J. P. Schermann, *Phys. Rev. Lett.* **73**, 2436 (1994); C. E. H. Dessent, C. G. Bailey, and M. A.

- Johnson, *J. Chem. Phys.* **103**, 2006 (1995); J. H. Hendricks, H. L. de-Clercq, S. A. Lyapustina, C. A. Fancher, T. P. Lippa, J. M. Collins, S. T. Arnold, G. H. Lee, and K. H. Bowen, in *Structures and Dynamics of Clusters*, Proceedings of the Yamada Conference XLIII, edited by T. Kondow (Universal Academy Press, Tokyo, 1995).
- ¹⁴O. H. Crawford, *Mol. Phys.* **20**, 585 (1971); C. Desfrancois, H. Adoul-Carime, N. Khelifa, J. P. Schermann, V. Brenner, and P. Millie, *J. Chem. Phys.* **102**, 4952 (1995).
- ¹⁵J. V. Coe, J. T. Snodgrass, C. B. Freidhoff, K. M. McHugh, and K. H. Bowen, *J. Chem. Phys.* **84**, 618 (1986).
- ¹⁶D. M. Neumark, K. R. Lykke, T. Andersen, and W. C. Lineberger, *Phys. Rev. A* **32**, 1890 (1985); M. J. Travers, D. C. Cowles, and G. B. Ellison, *Chem. Phys. Lett.* **164**, 449 (1989).
- ¹⁷J. V. Coe, J. T. Snodgrass, C. B. Freidhoff, K. M. McHugh, and K. H. Bowen, *J. Chem. Phys.* **87**, 4302 (1987).
- ¹⁸Examples include $(\text{H}_2\text{O})_2^+$, $(\text{HF})_2^+$, $(\text{H}_2\text{O})(\text{CH}_3\text{CN})^+$, $(\text{D}_2\text{O})(\text{CD}_3\text{CN})^+$, $(\text{H}_2\text{S})_4^+$, $(\text{H}_2\text{O})(\text{HCl})^+$, $(\text{H}_2\text{O})(\text{HCN})^+$, $(\text{H}_2\text{O})_3^+$, $\text{Ar}_n(\text{H}_2\text{O})_2^+$, and $(\text{EG})_2^+$, where EG=ethylene glycol.
- ¹⁹A. Les, L. Adamowicz, M. J. Nowak, and L. Lapinski, *Spectrochim. Acta* **48A**, 1385 (1992). Using the notation, QN, from this reference, peaks B–H in the uracil anion spectrum (Fig. 1) are assigned as the following molecular vibrations: B=Q28–Q26, C=Q21–Q18, D=Q13–Q11, E=Q8–Q7, F=Q6–Q5, G=Q4–Q3, and H=Q2–Q1, and peaks B–H in the thymine anion spectrum (Fig. 2) are assigned as B=Q34–Q30, C=Q27–Q22, D=Q19–Q17, E=Q13–Q10, F=Q9–Q7, G=Q6–Q3, and H=Q2–Q1.
- ²⁰C. G. Bailey, C. E. H. Dessent, M. A. Johnson, and K. H. Bowen, *J. Chem. Phys.* (submitted).
- ²¹F. W. Crow, K. B. Tomer, M. L. Gross, J. A. McCloskey, and D. E. Bergstrom, *Anal. Biochem.* **139**, 243 (1984); D. L. Slowikowski and K. H. Schram, *Nucleosides and Nucleotides* **4**, 347 (1985); K. J. Volk, M. S. Lee, R. A. Yost, and A. Brajter-Toth, *Anal. Chem.* **60**, 720 (1988); high resolution, negative ion mass spectral measurements by J. T. Snodgrass, using both electrospray and atmospheric pressure chemical ionization techniques.
- ²²During these searches, we did observe the photoelectron spectrum of $(\text{T-H})^-$. Its EA_a appears to be ~ 1.5 eV. We did not observe a comparable spectrum for $(\text{U-H})^-$ within our energy window.
- ²³R. N. Compton, H. S. Carman, C. Desfrancois, H. Adoul-Carime, J. P. Schermann, J. H. Hendricks, S. A. Lyapustina, and K. H. Bowen, *J. Chem. Phys.* (submitted).
- ²⁴J. R. Wiley, J. M. Robinson, S. Ehdaie, E. C. M. Chen, and W. E. Wentworth, *Biochem. Biophys. Res. Commun.* **180**, 841 (1991).
- ²⁵W. A. Bernhard, *Adv. Radiat. Biol.* **9**, 199 (1981); M. C. R. Symons, *J. Chem. Soc. Faraday Trans.* **83**, 1 (1987); W. A. Bernhard, *J. Phys. Chem.* **93**, 2187 (1989); D. Becker and M. D. Sevilla, *Adv. Radiat. Biol.* **17**, 121 (1993), and references therein.