

Bound anionic states of adenine

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Anionic states of nucleic acid bases are involved in DNA damage by low-energy electrons and in charge transfer through DNA. Previous gas phase studies of free, unsolvated nucleic acid base parent anions probed only dipole-bound states, which are not present in condensed phase environments, but did not observe valence anionic states, which for purine bases are thought to be adiabatically unbound. Contrary to this expectation, we have demonstrated that some thus far ignored tautomers of adenine, which result from enamine-imine transformations, support valence anionic states with electron vertical detachment energies as large as 2.2 eV, and at least one of these anionic tautomers is adiabatically bound. Moreover, we predict that the new anionic tautomers should also dominate in solutions and should be characterized by larger values of electron vertical detachment energy than the canonical valence anion. All of the newfound anionic tautomers might be formed in the course of dissociative electron attachment followed by a hydrogen atom attachment to a carbon atom, and they might affect the structure and properties of DNA and RNA exposed to low-energy electrons. The new valence states observed here, unlike the dipole-bound state, could exist in condensed phases and might be relevant to radiobiological damage. The discovery of these valence anionic states of adenine was facilitated by the development of (i) an experimental method for preparing parent anions of nucleic acid bases for photoelectron experiments, and (ii) a combinatorial/quantum chemical approach for identification of the most stable tautomers of organic molecules.

nucleic acid bases | photoelectron spectroscopy | tautomers | valence anion

Low-energy electrons are produced when high-energy radiation interacts with the contents of living cells. They might become trapped on nucleic acid bases (NBs) and trigger chemical transformations leading to DNA damage. Recent experiments suggested that single- and double-strand breaks develop in the DNA exposed to low-energy electrons (1). Furthermore, charged NBs play a critical role in electron and hole transfer in DNA (ref. 2 and references therein). Anionic states of NBs have been intensively studied both experimentally and theoretically. Purine bases, i.e., adenine (A) and guanine (G), are believed to have a much smaller affinity to an excess electron than pyrimidine bases (3–7). The reduction potentials of G and A are more negative than those of pyrimidines by a few tenths of an eV (3). The group of Burrow (4) reported an electron vertical attachment energy (VAE) of -0.54 eV for A and -0.46 eV for G, but -0.22 to -0.32 eV for pyrimidine bases. The negative values of VAE indicate that anions are electronically unbound at the equilibrium geometry of the corresponding neutral. An electron binding energy (EBE) of -0.45 eV for A was the smallest measured by Periquet *et al.* (5) among four NBs (U, T, A, and C). Li *et al.* (6) and Wesolowski *et al.* (7) predicted a negative adiabatic electron affinity (AEA) for the canonical tautomer of A. In conclusion, pyrimidine rather than purine bases have been considered as trapping sites for excess electrons in DNA, as illustrated in a series of computational studies (8–10).

Recent years brought, however, a revival of interest in anionic states of purine NBs (11–15). Ray *et al.* (11) demonstrated that

the probability of capturing of an excess electron increases with the number of guanines in short single and double strands of DNA. Harańczyk and Gutowski (12) identified several tautomers of guanine that support adiabatically bound valence anion. Mazurkiewicz *et al.* (14) predicted that adenine might become a favorable site for localization of an excess electron in the Watson–Crick AT pair providing that a weak acid coordinates to the Hoogsteen sites of adenine. Huber *et al.* (15) reported a high-resolution dissociative electron attachment result for gas phase adenine.

The results of our recent computational studies unraveled that the most stable valence anions of 1-methylcytosine (16), uracil (17), thymine (18), and guanine (12) are associated with tautomers that are unusual among neutral species. These so far ignored tautomers result from enamine-imine transformations of the canonical tautomer, i.e., a proton is transferred between the N and C atoms. We demonstrated that guanine, uracil, and thymine support adiabatically bound anions. The new and most stable tautomers of valence anions are characterized by electron vertical detachment energies (VDEs) that are typically 1.0–1.5 eV larger than those for valence anions of canonical tautomers. The stability of new anionic tautomers was initially counterintuitive, and their discovery was facilitated by a series of studies on intermolecular proton transfer reactions involving anions of NBs (19, 20, ††).

In this contribution we extend our computational studies to anionic tautomers of adenine and report photoelectron spectroscopy (PES) data for A^- . In these experiments, valence anions of A, i.e., A^- , are formed by irradiating an adenine film (supported on a translating, rotating metal rod) with low-power green light from a Nd:YAG laser. This source arrangement produces anion states that differ dramatically from those generated in our previous studies. In our earlier work, NBs were thermally evaporated in an argon-filled stagnation chamber before being expanded out a small nozzle. At that point, low-energy electrons from a filament were injected into the jet in the presence of a weak axial magnetic field, and the resultant NB anions were then extracted into the photoelectron spectrometer. Photoelectron spectra of parent NB anions formed in this way revealed them to be dipole-bound states (21–23), whereas photoelectron spectra of A^- generated by the laser irradiation

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Abbreviations: AEA, adiabatic electron affinity; CCSD(T), coupled cluster method with single, double, and perturbative triple excitations; EBE, electron binding energy; MP2, second-order Møller–Plesset; NB, nucleic acid base; PES, photoelectron spectroscopy; VAE, vertical attachment energy; VDE, vertical detachment energy.

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Table 1. The 10 most stable anionic tautomers of adenine identified in the course of prescreening 625 tautomers at the B3LYP/6-31+G level**

Order	Tautomer	AEA [†]	VDE [‡]
1	$aA_{2N4}^{C2,2C8}$	1.82	1.801
2	$aA_{2N4}^{2C2,C8}$	-0.44	2.196
3	$aA_{N4t}^{2C2,N3,C8}$	-1.97	2.603
4	$aA_{N4t}^{2C2,C4,C6}$ Z(C4,C6) [§]	-5.16	2.266
5	$aA_{2N4}^{N1,C2,C8}$	-6.04	0.621
6	$aA_{N4c}^{C2,N7,2C8}$	-6.35	2.533
7	$aA_{2N4}^{C2,C4,C8}$	-6.93	2.855
8	$aA_{N9,N4t}^{C2,2C8}$	-7.18	1.974
9	$aA_{N9,N4c}^{C2,2C8}$	-7.69	1.858
10	$aA_{N4t}^{C2,N3,2C8}$	-8.67	1.323

[†]Calculated with respect to the canonical tautomer, in kcal/mol. Not corrected for a contribution from zero-point vibrations.

[‡]In eV.

[§]Stereoisomer with the C4 and C6 hydrogens on the same side of the molecular plane.

method described above showed them to be valence anion states with no evidence for the presence of dipole-bound anions. In this report we demonstrate that adenine supports several anionic states, which are at least vertically bound with respect to the neutral, i.e., are characterized by positive values of VDE. Moreover, we predict the existence of at least one enamine-imine tautomer, which is adiabatically bound with respect to the most stable neutral tautomer, although the stability, ≈ 1 kcal/mol, is smaller than for anionic enamine-imine tautomers of guanine (12). The valence-state species observed here, unlike the dipole-bound state, could potentially exist in water solutions and biological cells, and therefore might be relevant to radiobiological damage.

Results and Discussion

The 10 most stable anionic tautomers identified in the prescreening process, i.e., at the B3LYP/6-31+G** level of theory, are presented in Table 1. The most stable tautomer, $a_{val}A_{2N4}^{C2,2C8}$ (for its structure see Fig. 1) is adiabatically bound by 1.82 kcal/mol with respect to the canonical neutral $A_{N9,2N4}^{C2,C8}$. The next most stable anionic tautomers are $a_{val}A_{2N4}^{2C2,C8}$ and $a_{val}A_{N4t}^{2C2,N3,C8}$, which are less stable than $A_{N9,2N4}^{C2,C8}$ by 0.44 and 1.97 kcal/mol, respectively. Among the biologically relevant tautomers, i.e., with an H on N9, the most stable proved to be $a_{val}A_{N9,N4t}^{C2,2C8}$ and

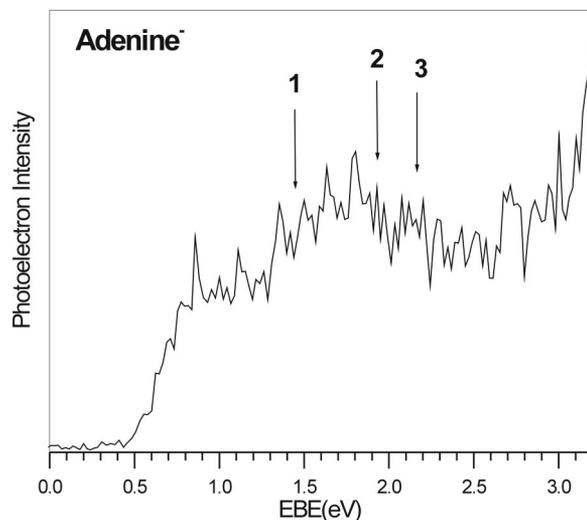


Fig. 2. Photoelectron spectrum for A^- . The calculated VDE of first three most stable adenine tautomers are marked by arrows. 1, the most stable tautomer, $a_{val}A_{2N4}^{C2,2C8}$. 2, the second most stable tautomer, $a_{val}A_{2N4}^{2C2,C8}$. 3, the third most stable tautomer, $a_{val}A_{N4t}^{2C2,N3,C8}$.

$a_{val}A_{N9,N4c}^{C2,2C8}$, which are the eighth and ninth most stable anionic tautomers. The former cannot maintain the Watson-Crick set of hydrogen bonds with thymine, but the latter can. These five anionic tautomers were further refined at the second-order Møller-Plesset (MP2) and CCSD(T) (coupled cluster method with single, double, and perturbative triple excitations) levels of theory. Here we do not report on the valence anion of the canonical tautomer, $A_{N9,2N4}^{C2,C8}$, because at the MP2 level of theory we could not identify a minimum on the potential energy surface, at which the valence anion would be vertically bound with respect to the neutral.

The refined results, obtained at the CCSD(T)/AVDZ//MP2/AVDZ level (AVDZ, augmented, polarized correlation-consistent basis set of double-zeta quality), are summarized in Fig. 1. $a_{val}A_{2N4}^{C2,2C8}$ remains adiabatically bound with respect to $A_{N9,2N4}^{C2,C8}$ by 0.88 kcal/mol and is characterized by a VDE of 1.43 eV (see Fig. 2). The corresponding neutral structure, $A_{2N4}^{C2,C8}$, was found to be 28.46 kcal/mol less stable than $A_{N9,2N4}^{C2,C8}$ in terms of electronic energy corrected for zero-point vibrations. The anions, $a_{val}A_{2N4}^{2C2,C8}$ and $a_{val}A_{N4t}^{2C2,N3,C8}$ were found adiabatically un-

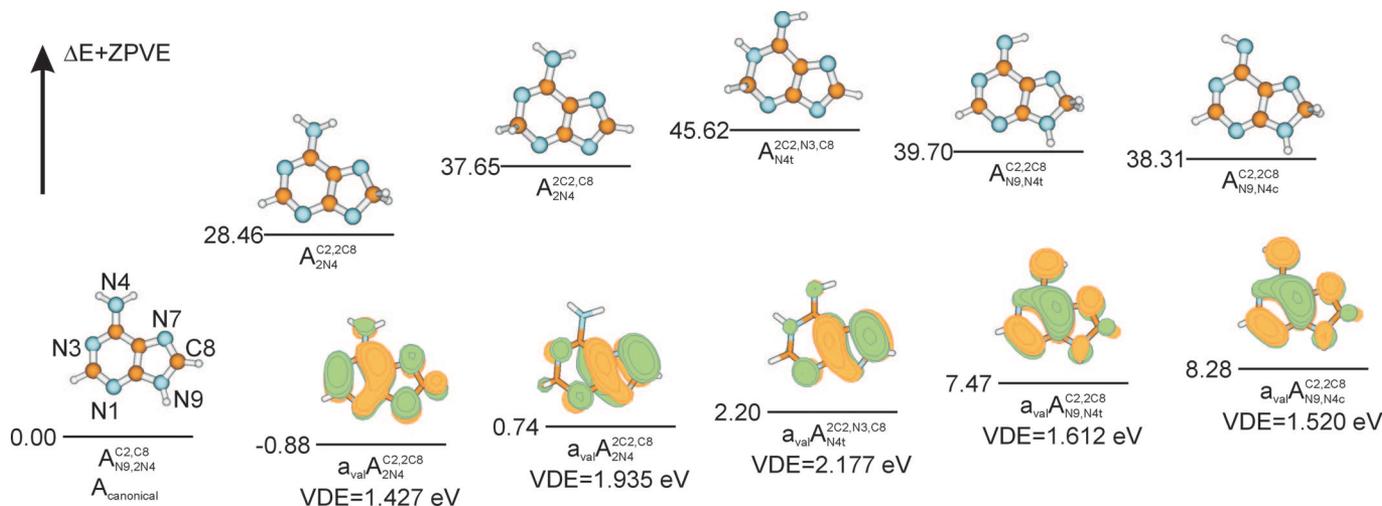


Fig. 1. Relative electronic energies corrected for energies of zero-point vibrations ($\Delta E + \text{ZPVE}$ in kcal/mol) and electron vertical detachment energies (VDE in eV).

Table 2. AEA's (calculated with respect to the canonical tautomer, in kcal/mol) and electron VDEs (in eV) for the tautomers of hydrated adenine characterized within the polarizable continuum model

Tautomer	AEA [†]	VDE [‡]
$aA_{N9,2N4}^{C2,C8}$	33.18	2.195
$aA_{2N4}^{C2,C8}$	45.91	4.216
$aA_{2N4}^{C2,C8}$	43.39	4.642
$aA_{N4t}^{2C2,N3,C8}$	41.71	5.019
$aA_{N9,N4t}^{C2,C8}$	39.68	4.335
$aA_{N9,N4c}^{C2,C8}$	39.89	4.424

[†] $\epsilon = 78$.

[‡] $\epsilon = 78$ and 2 for the initial and final state, respectively.

bound by 0.74 and 2.20 kcal/mol but vertically bound by 1.94 and 2.18 eV, respectively (see Fig. 2). The biologically relevant anions, $a_{\text{val}}A_{N9,2N4}^{C2,C8}$ and $a_{\text{val}}A_{N9,N4c}^{C2,C8}$, are less stable and adiabatically unbound with respect to $A_{N9,2N4}^{C2,C8}$ by 7.47 and 8.28 kcal/mol, respectively. The corresponding VDEs of 1.61 and 1.52 eV are only slightly larger than the VDE of $a_{\text{val}}A_{2N4}^{C2,C8}$. If the anionic beam containing A^- could reach equilibrium, then the adiabatically bound tautomer, $a_{\text{val}}A_{2N4}^{C2,C8}$, should dominate in the gas phase because the AEA of the dipole-bound anion of $A_{N9,2N4}^{C2,C8}$ was measured to be only 0.28 ± 0.11 kcal/mol (24). Formation of the enamine-imine anionic tautomers might, however, require overcoming barriers the heights of which are tautomer dependent. For this reason, experimental results on gas phase anions of adenine might strongly depend on their formation conditions.

The photoelectron spectrum of adenine anion was measured with 3.493 eV photons, and the result is presented in Fig. 2. A broad band (or a combination of bands) begins from ≈ 0.5 eV and continues unabated up through the maximum EBE of the spectrum. Because a dipole-bound anion state has a distinctive photoelectron signature, wherein its spectrum is dominated by a single narrow peak at very low EBE (21), the spectrum of adenine anion presented here is clearly not that of a dipole-bound state and in fact is due to a valence-bound state or states of the adenine anion. The calculated VDE values for the three most stable anionic tautomers are marked by vertical bars in Fig. 2 and are spread over the broad band. The observed broad band spectral congestion is probably due to the simultaneous presence of several tautomers of the adenine anion (see Fig. 1).

How relevant are our findings about new anionic tautomers for solvated species? It is known that anions of NBs become protonated in water solutions (see ref. 25 and references therein, and ref. 26). Here we consider only the effect of electrostatic stabilization by water using the polarizable continuum model, and the results are presented in Table 2. The anion of canonical tautomer $a_{\text{val}}A_{N9,2N4}^{C2,C8}$ is adiabatically bound by 33.2 kcal/mol. However, the new enamine-imine tautomers are more stable than $a_{\text{val}}A_{N9,2N4}^{C2,C8}$ by at least 6 kcal/mol. The enamine-imine anionic tautomers have much larger VDEs than the canonical tautomer, a feature that is amenable to experimental verification. We believe that the new anionic tautomers will dominate not only in the gas phase but also in solvents, and we suggest experimental studies in aprotic solvents to verify our predictions.

What might be formation pathways for the new anionic tautomers? For analogous tautomers of pyrimidine bases the barriers for intramolecular proton transfer are prohibitively large at standard conditions (12, 16–18). We suggest two formation pathways of the new anionic tautomers. First, they might be formed through intermolecular proton transfer (27, 28). Second, dissociative electron attachment (15) might facilitate their formation. Let us consider formation of $a_{\text{val}}A_{N9,2N4}^{C2,C8}$ starting from the neutral $A_{N9,2N4}^{C2,C8}$ scattering target:

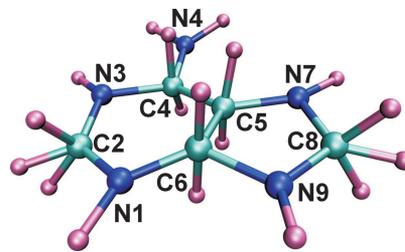
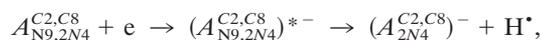
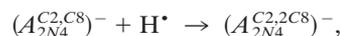


Fig. 3. Molecular frameworks of adenine with all sites considered for hydrogen attachment.



where $(A_{N9,2N4}^{C2,C8})^{*-}$ denotes a scattering state for an excess electron and $(A_{2N4}^{C2,C8})^-$ denotes a deprotonated adenine in the ground electronic state. The attachment of a hydrogen atom to C8,



encounters a small barrier of 7.3 kcal/mol calculated at the MP2/6–31+G** level. The situation is even more favorable for other enamine-imine tautomers, because the attachment of H to C2 (to form $a_{\text{val}}A_{2N4}^{2C2,C8}$ and $a_{\text{val}}A_{N4t}^{2C2,N3,C8}$) and to C8 (to form $a_{\text{val}}A_{N9,N4t}^{C2,2C8}$ and $a_{\text{val}}A_{N9,N4c}^{C2,2C8}$) is barrierless. Thus, kinetics rather than thermodynamic factors might indeed control the tautomeric composition of A^- in the beam.

Conclusion

In summary, adenine supports at least one adiabatically bound valence anion, which results from an enamine-imine transformation, with a proton being transferred from N9 to C8. The small but positive AEA of 0.88 kcal/mol predicted at the CCSD(T)/AVDZ level of theory might become larger after the basis set limiting values are obtained (18). We predict that this anionic tautomer will dominate in the equilibrated anionic beam, because the AEA of the dipole-bound anion based on the canonical tautomer was predicted to be only 0.28 ± 0.11 kcal/mol (24). The computed values of VDE for the enamine-imine tautomers identified in this study are within the broad range of the dominant PES feature for A^- . It clearly shows that the characteristics of biomolecular anions are highly dependent on their tautomeric form, and the distribution of the different possible tautomers is strongly dependent on the method of generation of anionic species. The discovery of these valence anionic states of adenine was facilitated by the development of (i) an experimental method for preparing parent anions of NBs for PES experiments, and (ii) a combinatorial/quantum chemical approach for identification of the most stable tautomers of organic molecules. The role of the new tautomers in chemical transformations of DNA and in the charge transfer through DNA still needs to be explored.

Materials and Methods

The computational search for stable valence anions of A was performed according to a procedure successfully applied by us for guanine (12) and further extended in ref. 29 (TauTGen, <http://tautgen.sf.net>). This procedure consists of three steps: (i) combinatorial generation of an extended library of tautomers; (ii) prescreening the tautomers at the density functional level of theory with a B3LYP exchange-correlation functional (30–32) and 6–31+G** basis set (33, 34); and (iii) the final geometry, zero-point vibrational energy, and electronic energy refinements for the top hits from step ii. The geometries and harmonic frequencies of the top hits are determined at the MP2 and their final electronic energies at the CCSD(T) level (35). An aug-

mented, polarized correlation-consistent basis set of double-zeta quality (AVDZ) (36) has been used in the MP2 and CCSD(T) calculations. The library of tautomers was generated with the program TauTGen 0.03 (29). TauTGen builds all possible tautomers from a molecular framework (the core) and a specified number of hydrogen atoms. The hydrogens are attached to the predefined sites specified by a user. The resulting structures reflect various hybridizations of core atoms and include rotamers and stereoisomers. The library of tautomers is combinatorially generated within a user-defined list of constraints on the minimum and maximum number of hydrogens at each heavy atom. We defined 20 sites of possible hydrogen attachment (see Fig. 3), and we imposed the following constraints: 0 or 1 hydrogen atom on N1, N3, C4, C5, C6, N7, and N9; 1 or 2 hydrogen atoms on N4; 0, 1, or 2 hydrogens on C2 and C8. With these constraints we generated 625 initial structures including the E, and Z isomers of tautomers having at least two hydrogens at any of C4, C5, and C6, and rotamers of the N4 imino group. They were divided into two groups: those that have a hydrogen atom at N9 (i.e., might be biologically relevant) and those that do not. In addition, each group was divided into three subgroups: 2N4 (amino tautomers), N4c, and N4t (imino-tautomers with a hydrogen at N4 in the cis and trans position with respect to N3, respectively). We use a notation A_a^b , where “a” is a group and subgroup and “b” is a string of heavy atoms the remaining hydrogen atoms are connected to. The anionic species bear a prefix a_{val} (see Fig. 1). The effects of hydration were included within the integral equation formalism-polarizable continuum method with the cavity built up by using the United Atom (UA0) model (37). The calculations were performed by using Gaussian 03 (Revision C. 02; Gaussian, Wallingford, CT) and Molpro (a package of *ab initio* programs designed by H.-J. Werner and P. J. Knowles; Version 2002.1) software, and molecular structures and orbitals were drawn with the program MOLDEN (38).

Negative ion PES was conducted by crossing a mass-selected beam of negative ions with a fixed frequency photon source and energy analyzing the resultant photodetached electrons. This technique is governed by the energy-conserving relationship $h\nu = \text{EKE} + \text{EBE}$, where $h\nu$ is the photon energy, EKE is the

measured electron kinetic energy, and EBE is the electron binding energy. Briefly, both mass spectra and photoelectron spectra were collected on an apparatus consisting of a laser vaporization source employing a Nd:YAG laser, a linear time-of-flight mass spectrometer for mass analysis and selection, a second Nd:YAG laser used for photo-detachment, and a magnetic bottle used for electron energy analysis. The details of our apparatus have been described elsewhere (39).

As mentioned above, adenine anions were generated in these experiments by using a laser vaporization ion source. This source consisted of a rotating, translating adenine-coated metal rod (Cu or Ag), a laser beam entrance port, a pulsed gas valve to feed pulses of helium into the laser-sample interaction region, and a gas expansion exit nozzle. Typically, helium gas at 4 bar was expanded in synchronization with laser ablation pulses. The adenine-coated rods were prepared by pressing adenine powder directly onto the metal rod to form a thin layer on its surface. The adenine coating was then ablated at very low laser power with the second harmonic (532 nm) of a Nd:YAG laser. We speculate that the role of the metal rod was to supply photoemitted electrons. No mixed metal/adenine cluster anions were observed.

The calculations were performed at the Academic Computer Center in Gdańsk, the National Energy Research Scientific Computing Center, and the Molecular Science Computing Facility of the William R. Wiley Environmental Molecular Sciences Laboratory, a national scientific user facility sponsored by the U.S. Department of Energy Office of Biological and Environmental Research and located at Pacific Northwest National Laboratory, which is operated by Battelle for the U.S. Department of Energy. The Molecular Science Computing Facility resources were available through a Computational Grand Challenge Application grant. The computational portion of this work was supported by Polish State Committee for Scientific Research Grants DS/8000-4-0140-7 (to M.G.) and N204 127 31/2963 (to M.H.), European Social Funds ZPORR/2.22/II/2.6/ARP/U/2/05 (to M.H.), and the U.S. Department of Energy Office of Biological and Environmental Research, Low Dose Radiation Research Program (M.G.). M.H. holds the Foundation for Polish Science award for young scientists. The experimental material in this paper (that of K.H.B.) is based on work supported by National Science Foundation Grant CHE-0517337.

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