Excess Electron Attachment to the Nucleoside Pair 2′-Deoxyadenosine (dA)—2′-Deoxynthymidine (dT)

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Supporting Information

ABSTRACT: The 2′-deoxyadenosine···2′-deoxynthymidine (dAdT**−) radical anion nucleoside pair has been investigated both experimentally and theoretically in the gas phase. The vertical detachment energy (VDE) and adiabatic electron affinity (AEA) were determined by anion photoelectron spectroscopy (PES). The measured photoelectron spectrum features a broad band having an onset at ~1.1 eV and a maximum at the electron binding energy (EBE) ranging from 1.7 to 1.9 eV. Calculations performed at the M06-2X/6-31++G** level reveal that the observed PES signal is probably due to a dAdT**− complex in which the thymine of the dT nucleoside forms hydrogen bonds that engage its O7 and O8 atoms as well as the 3′- and 5′-hydroxyl groups of 2′-deoxyadenosine (dA), while dT’s 3′-hydroxyl group interacts with the N1 of dA. In this heterodimer, the excess electron is entirely located on thymine. The biologically relevant Watson–Crick arrangement of the dAdT**− dimer was found to be substantially less stable (by ~19 kcal mol−1 in Gibbs free energy scale) than the above-mentioned configuration; hence, it is not populated in the gas phase.

INTRODUCTION

An interest in the anionic states located in DNA was stimulated by the finding that low-energy electrons are capable of introducing single- (SSB) and double-strand breaks (DSB) to DNA.1 The experimental investigations that followed this finding were accompanied by computational studies related to the molecular details of the electron-induced damage. In particular, theoretical works comprised electron interactions with a variety of systems starting from single nucleobases and ending up with the single-stranded and double-stranded nucleotide oligomers.1−7

Early theoretical studies suggested that the formation of stable valence-type anions of nucleobases is unlikely in the gas phase,5−12 while several spectroscopy experiments demonstrated that the isolated pyrimidines exist as dipole-bound rather than valence-type anion radicals.3−15 However, it turned out that nucleobases form adiabatically bound anions due to even marginal solvation by such species as xenon, water, or methanol.14−18 The biologically relevant complementary base pairs, AT and GC, may be perceived as systems in which the pyrimidine base is solvated by purine, so they should form stable valence-type anion radicals where the excess electron is localized to T or C. As expected, the gas-phase anions of AT15 and GC10 have been found by negative ion photoelectron spectroscopy, which also confirmed the results of other experimental techniques.21,22 Computational reports on AT23−28 and GC23,28−31 greatly outnumbered the experimental reports.

The anions of nucleobases themselves are also viable in the gas phase provided they are chemically modified. On the basis of DFT calculations for 2′-deoxynucleosides, Richardson et al.32 predicted that the T and C pyrimidines when substituted with 2′-deoxyribose at the N1 site should form a valence-type anion in the gas phase. In contrast, the computed adiabatic electron affinity (AEA) and vertical detachment energy (VDE) values close to zero for 2′-deoxyguanosine (dG**) do not support the existence of a valence-type anion of this purine-based nucleoside, whereas the computational results for 2′-deoxyadenosine (dA**) were ambiguous (unfavorable AEA was found in contrast to favorable VDE). Stokes et al.,33 employing a combination of infrared desorption, electron photoemission, and gas jet expansion, was not successful in the generation of a stable anion radical of dG. On the other hand, they recorded the anion photoelectron spectra of 2′-deoxyadenosine (dA). These authors also measured the photoelectron spectrum of the ribonucleoside parent anion of guanine**− (rG**) and three other ribonucleosides: uridine**− (rU**−), cytidine**− (rC**−), and adenosine**− (rA**−).33

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Nucleotides are also able to effectively capture electrons. Electron attachment to the isolated nucleotides was widely studied in the context of DNA damage, while less attention was paid to the nondissociative generation of parent anion radicals of nucleotides in the gas phase.

More recently, photoelectron spectroscopy allowed for the investigations of relatively large anionic systems as homodimeric nucleoside pairs, uridine (U•−), thymidine (T•−)2, and cytidine (C•−)2. The PES studies combined with DFT calculations demonstrated that such nucleoside homodimers exhibit substantially increased tendency to attach an electron in comparison to single nucleosides and appear in the gas phase as stable complexes in which the excess electron resides entirely on the pyrimidine moiety of a single nucleoside.

In the present study, we explore the intrinsic electrophilic properties of the biologically relevant nucleoside pair 2′-deoxyadenosine–2′-deoxycytidine (dAdT). In the PES experiment, we have observed formation of the stable valence anion of dAdT. From the measured spectrum, we have extracted the respective AEA and VDE values. Since the PES technique does not provide any details about the conformation of registered anions, for characterization of the geometries of species existing in the experimental conditions, we took advantage of a computational approach. Based on DFT calculations, we have identified the structure of dAdT•−, which is responsible for the PES feature. The theoretical AEA and VDE obtained for this heterodimer reproduce the experimental values very well.

■ METHODS

Experimental Details. The anion radical dimers of dAdT were generated using a pulsed infrared desorption–pulsed visible photoemission anion source. Anion photoelectron spectroscopy (PES) is conducted by crossing beams of mass-selected negative ions and fixed frequency photons, followed by energy analysis of the resulting photodetached electrons. Spectral bands in the spectrum result from the vertical Franck–Condon overlap between the wave function of the anion and the wave function of the resulting neutral. This technique is based on the energy conserving relationship $hν = EBE + EKE$, where $hν$ is the photon energy, EBE is the electron-binding energy, and EKE is the measured electron kinetic energy. The EBE corresponding to the intensity maximum on the spectrum is referred to as the vertical detachment energy. In the present study, we explore the intrinsic electrophilic properties of the biologically relevant nucleoside pair 2′-deoxyadenosine–2′-deoxycytidine (dAdT). In the PES experiment, we have observed formation of the stable valence anion of dAdT. From the measured spectrum, we have extracted the respective AEA and VDE values. Since the PES technique does not provide any details about the conformation of registered anions, for characterization of the geometries of species existing in the experimental conditions, we took advantage of a computational approach. Based on DFT calculations, we have identified the structure of dAdT•−, which is responsible for the PES feature. The theoretical AEA and VDE obtained for this heterodimer reproduce the experimental values very well.

RESULTS AND DISCUSSION

The photoelectron spectrum of the 2′-deoxyadenosine–2′-deoxycytidine radical anion is presented in Figure 1. It consists of a broad peak with the maximum between 1.7 and 1.9 eV, which corresponds to the experimental VDE value. The adiabatic electron affinity may be estimated as corresponding to the EBE value at ~10% of the rising photoelectron intensity. Thus, from the onset of the photoelectron spectrum, the AEA for dAdT•− can be estimated to be ~1.2 eV.

Photoelectron spectra were calibrated against the well-known photoelectron spectrum of Cu•−. The adiabatic electron affinities, AEA and AEAo, are defined as the difference between the electronic energies and Gibbs free energies, respectively, of the neutral and the anion at their fully relaxed geometries (AEA = $E(\text{neu}+\text{an}) - E(\text{an}+\text{neu})$). All pictures of structures and molecular orbitals were plotted with the GaussView 5.0 program.
An electron attachment to the Watson–Crick dAdT nucleoside pair was studied computationally by Gu et al. Their B3LYP/DZP++ results unequivocally confirmed that the dAdT complex, being characterized by a positive vertical electron affinity of 0.2 eV and the adiabatic electron affinity equal to 0.6 eV, is able to form a stable anion in vacuum. At this level of theory, vertical detachment energy of 1.14 eV was obtained for dAdT**. However, Gu’s VDE and AEA values do not reproduce the experimental values of the current report. Since their approach was demonstrated to be appropriate for calculating the energetics of electron attachment to the nucleobase related system, it seems unlikely that such a large discrepancy between Gu’s predictions and our measurements may result from the deficiency of the computational model. Indeed in the following, we demonstrate that the PES discrepancy between Gu’s B3LYP/DZP++ results unequivocally contra the existence of a stable valence radical, our search for the dimeric anion radicals responsible for the measured PES feature involved possible arrangements of the dAdT** dimer composed of fully optimized monomers, utilizing 2'-deoxyadenosine in its relaxed neutral geometry and 2'-deoxythymidine in its relaxed anionic geometry.

In the scrutinized geometries of the dAdT**, particular monomers interact via their proton-acceptor (O7(T), O8(T), N1(A), N3(A)) and proton-donor (O3’H, O5’H, N6H(A), C2H(A), N3H(T)) sites. Thus, we obtained 26 structures, shown in Figure 3, which according to the type of the hydrogen bond pattern can be categorized into six groups. In the five out of six groups only the thyminyl moiety of 2’-deoxythymidine participates in hydrogen bonding with 2’-deoxyadenosine. The first family consists of heterodimers, where hydrogen bonds are formed solely between nucleobases ("A···T") family. In the second group, labeled as "A···T/sugar···T", thymine interacts (utilizing its proton-acceptor and hydrogen-donor sites) with both sugar and adenine of dA. In the third family, "sugar···T", the thyminyl moiety forms one or two hydrogen bonds with the hydroxyl groups of the sugar of dA. The next group

<table>
<thead>
<tr>
<th>Table 1. Adiabatic Electron Affinities (eV) and Vertical Detachment Energies (eV) of 2’-Deoxynucleosides and Canonical Nucleoside Pair dAdT, Calculated at the M062X/6-31++G**(level) along with singly occupied molecular orbital plotted with a contour value of 0.05 b**.</th>
<th>AEA_E</th>
<th>AEA_A</th>
<th>VDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>dA</td>
<td>-0.16</td>
<td>-0.17</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>0.06^a</td>
<td></td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>-0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dT</td>
<td>0.30</td>
<td>0.33</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>0.44^a</td>
<td></td>
<td>0.94</td>
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<tr>
<td></td>
<td>0.45^a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dAdT</td>
<td>0.43</td>
<td>0.46</td>
<td>1.18</td>
</tr>
<tr>
<td></td>
<td>0.60^a</td>
<td></td>
<td>1.14</td>
</tr>
</tbody>
</table>

^aReference 32.  Reference S1.  Reference S0.
“sugar⋯T/stack” comprises only two structures. In these complexes in addition to single thymine⋯sugar hydrogen bond, \( \pi \)-stack interactions are present and the distances between atoms of the overlapping aromatic T and A rings are in the range from \( \sim 3 \) to \( \sim 4 \) Å. To this end, we have also obtained a heterodimer arrangement in which nucleosides are complexed mainly due to the stacking interactions (see the structure labeled “stack” in Figure 3). The last group features an antiparallel (head-to-tail) orientation of nucleosides, that is, each 2′-deoxyribose is hydrogen-bonded to the nucleobase of the other monomer (the family labeled “sugar⋯T/A⋯sugar”). In case of three heterodimers from this group (20, 20′, and 21), cohesive forces between nucleosides result from H-bonds and dispersion interactions between aromatic rings of the nucleobases.

A common feature of the studied complexes is that the excess electron is localized on the \( \pi^* \) orbital of the thymine moiety, and the SOMO orbital closely resembles that of the valence anion of isolated thymine.\(^{52,53}\) The excess electron is located at the C4⋯C5 bond and at the C6 and O8 atoms of thymine. A

Figure 3. Structures of anion radical heterodimers \( \text{dAdT}^{\cdot-} \) optimized at the M06-2X/6-31++G*** level.
smaller amount of the excessive charge also resides on the nitrogen atoms, N1 and N3. Therefore, the investigated anionic complexes can be perceived as the thymidine valence anion solvated by the neutral adenosine. The heterocyclic ring of the thymine valence anion undergoes distortion, which diminishes destabilizing effects present in the singly occupied antibonding π* orbital of thymine (see Figure 3 and Figure S1).

The thermodynamic characteristics obtained at the M06-2X/6-31+G** level for the anionic radicals of dAdT heterodimers are summarized in Table 2. The relative stabilities (ΔG) of the studied configurations suggest that the PES spectrum results from electron detachment from structure 22 belonging to the "sugar···T/A···sugar" family. In this dimer, 2'-deoxythymidine is complexed to 2'-deoxyadenosine by three hydrogen bonds: two between the sugar of dA and thymine, 3'OH···O7 and S'OH···O8, and one between the sugar of dT and adenine, 3'OH···N1. Structure 22 is characterized by AEAG and VDE of 1.08 and 1.77 eV, respectively, which perfectly reproduces the experimental values of ~1.2 and 1.7–1.9 eV, respectively. Configuration 22 possesses the highest AEAG among all the considered complexes. In summary, we have predicted a positive AEAG for all conformers.

A large difference in the Gibbs free energies (nearly 19 kcal/mol) was found between the value of structure 22 and the canonical Watson–Crick arrangement of dAdT” (dimer 1). The second most stable anion radical is 14 from the "sugar···T" group. In this complex, similarly to 22, the thymine is involved in two hydrogen bonds with the 2'-deoxyribose of dA. The difference between both structures is that thymine in 14 utilizes different carbonyl oxygens than thymine in 22 to bind to the sugar (in 14 there are 3'OH···O8 and S'OH···O7 interactions). Moreover, as opposed to structure 22, dimer 14 does not possess a third hydrogen bond between thymidine’s sugar and adenine. The difference, in terms of Gibbs free energy, between 22 and 14 is large, 4.8 kcal/mol, which ensures that the configuration 14 is not populated in the gas phase. It can also be noted that VDE of 2.03 eV predicted for 14 is slightly off with respect to the maximum of the PES spectrum. The involvement of both proton-acceptors, O7 and O8, of T with both proton-donating hydroxyl groups of the sugar in dA occurs only in 14 and 22. Structurally closest to them is 13, where both hydroxyl groups of the adenosine’s sugar are involved in binding to thymine. Here, we have found in addition to 3'OH···O8, a quite unusual hydrogen bond, S'OH···N3. This structure, however, despite its VDE value matching well the experimental VDE, is insignificant according to relative thermodynamic stability criterion (it is higher by 7.8 kcal/mol on the Gibbs free energy scale than dimer 22). The next most stable, with respect to 22, dimeric configurations, 14, 19, 20, and 13 belong to "sugar···T" and "sugar···T/A···sugar" groups and their common feature is a 3'OH···O8 bond. The previous studies of thymidine (dT2ff) and uridine (dU2ff) homodimer anion radicals demonstrated that such 3'OH···O8 hydrogen bond strongly stabilizes anionic complexes of pyrimidine nucleosides and renders them viable in the gas phase. As can be seen from the data shown in Table 2, in addition to the 3'OH···O8 interaction involving adenosine’s sugar, the S'OH···O8 hydrogen bond is also important. However, in contrast to structures with 3'OH···O8 bonding (10–12a), we have not found a dimer featuring a single S'OH···O8 bond, and this bond in heterodimers is always accompanied by other interactions (see structures 9, 15, 16, 21, and 22).

We observe the weakest stabilization of the excess electron when nucleosides form dimers employing available centers of nucleobases ("A···T" family). Members of this family are characterized by the lowest electron affinities and VDE values among the complexes in Table 2. For dimers 1–4, we obtained VDE near 1 eV, which is close to the VDE found for isolated dT, ~0.9 eV. There is also similarity in AEAG values between 1–4 and dT. In the discussed "A···T" group, there are also two analogues of structure 1, labeled 1pt and 1a, which exhibit higher VDEs than the parent dimer, 1.79 and 1.44 eV, respectively. In case of 1pt an intermolecular proton transfer (PT) from the amine group of dA to O8 of the thyminyl moiety is responsible for such an increase in the calculated VDE. A proton transfer, often barrierless, was suggested in the past to be involved in a number of anionic nucleobase related complexes in the gas phase. As regards a complex of isolated nucleobases adenine and thymine, the previous PES/computational studies revealed that in the most stable complex of AT, electron induced proton transfer takes place from N9H of adenine to O8 of thymine. It was shown that PT increases the VDE of given anion radical by 0.7 eV compared with the non-PT structure with identical H-bond pattern. Interestingly,
among potential proton-donating centers, only the N9H center of adenine exhibits sufficient acidity to protonate thymine, and the methylation of the N9 atom prevents PT in the AT** complex. More recently, we have discovered PT in homodimers consisting of thymidine nucleosides (dT2)**. PT from N3H to C6 in 2'-deoxythymidine in structure 1a, which allows for a better delocalization of the excess charge within dT. Complexes 1 and 1a feature an identical intermolecular bond pattern between nucleosides but differ with respect to the intrinsic conformation of dT. The VDE value calculated for structure 1a is larger by 0.26 eV than that calculated for 1. The effect of such intramolecular interaction in dT can also be analyzed by comparing structure 12 with 12a (see Figure 3 and S1). The increase of VDE in 12a is similar to that found for 1a.

From the relative stabilities of the anion radical structures of dAdT scrutinized herein, the general picture emerges that the gaseous 2'-deoxyribonucleosides arrange themselves according to the dipole (adenosine moiety)···monopole (thymine moiety) interactions. Inspection of the SOMOs of all structures shown in Figure S1 reveals that the excess electron within dAdT** is attracted mainly by the thymyl moiety. Natural population analysis performed earlier by Gu et al. for dAdT** in the Watson–Crick arrangement indicates that the excess electron is distributed among adenine, thymine, and thymine’s 2'-deoxyribose in the proportion 0.07, 0.81 and 0.12, respectively. Thus, all dAdT** structures studied here may be perceived as the complexes of the neutral dA and anionic dT. Such complexes exhibit the strongest stability when the positive pole of the 2'-deoxyadenosine’s dipole, which is located in the sugar moiety, attracts the thymyl moiety, which hosts most of the excess electron. Thus, the low relative stability of the “A···T” family structures may result, at least partially, from the repulsion between negative pole of the 2'-deoxyadenosine’s dipole and the excess charge located on thymine.

**CONCLUSIONS**

Negative ion photoelectron spectroscopy was used to study a parent anion radical of a nucleoside pair between 2'-deoxyadenosine and 2'-deoxythymidine. The measured adiabatic electron affinity and vertical detachment energy of dAdT** are ~1.2 and 1.7–1.9 eV, respectively. The position of the signal (corresponding to experimental VDE values) as well as its width indicate a strong stabilization of dAdT upon electron attachment and the formation of valence-bond-type anions. Complementary computational investigations allowed for identification of the structure of dAdT that forms in the gas phase under PES experimental conditions. The calculated AEA and VDE for this heterodimer agree well with the measured values. This report makes a combined experimental and computational contribution to our understanding of the electrophilic properties of DNA components.

**REFERENCES**


**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcb.6b03450.

Large version of Figure 3 with optimized structures of anion radical heterodimers dAdT** supplemented with their singly occupied molecular orbitals plotted with a contour value of 0.05 b−3/2, complete refs 5 and 42, and cartesian coordinates of the optimized dAdT** heterodimers shown in Figures 3 and S1 (PDF).


