Unexpectedly Small Effect of the DNA Environment on Vertical Ionization Energies of Aqueous Nucleobases

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Abstract: By a combination of *ab initio* calculations and photoelectron spectroscopy we demonstrate that the DNA surrounding has only a negligible effect on ionization energies of nucleobases in the native aqueous environment. The aqueous solution thus effectively screens the sugar-phosphate backbone and the neighboring nucleobases. Consequently, vertical ionization potentials of nucleobases in aqueous DNA can be reliably derived from the corresponding values for its building blocks in water, i.e., aqueous nucleobases, nucleosides, or nucleotides.

Direct radiation-induced damage of DNA is initiated by ionization of nucleobases, which then may be followed by hole migration along the double helix.[1] Despite the importance of this process and its role in mutation or cell death as exploited in radiation treatment of cancer,[2] the ionization energies of DNA in its native aqueous environment are not firmly established. Only estimates of threshold ionization energies of selected DNA components were available in the literature,[3, 4] together with X-ray absorption data on band gap energies for supported dry DNA films.[5] Recently, photoelectron spectroscopy (PES) combined with the technique of liquid microjets has allowed for experimental determination of the lowest vertical ionization energies (VIE) of aqueous pyrimidine nucleosides.[6] The corresponding *ab initio* calculations[6, 7] showed that, unlike in the gas phase,[8-10] the values of VIEs are essentially the same for a given aqueous nucleobase and its nucleoside or nucleotide. In other words, we found that water with its high dielectric constant effectively screens the effects of the addition of the (deoxy)ribose sugar and phosphate on the ionization of the nucleobase.[6, 7]

From this perspective, it is compelling to address the issue of ionization of DNA in its native aqueous environment. Unfortunately, there have been no direct experimental data and quantum mechanics/molecular mechanics (QM/MM) calculations have provided conflicting evidence.[11, 12] The two papers described similar computational setups, nevertheless, the former calculations[11] pointed to a modest environmental influence, while the latter[12] to a strong effect of the DNA environment on the VIE of nucleobases in water. As a matter of fact, the latter study predicted that the fully hydrated DNA environment increases the VIEs of nucleobases by more than 3 eV.[12] The authors state that this large shift "is created by the complex molecular environment of DNA and that the interactions with the solvent structure around the DNA-cation play a key role". Further they attribute the difference from results of Ref. 11 to different computational details in accounting for the molecular mechanics environment.[12] Such a dramatic environmental effect would, however, put many hole-transfer calculations[13] in doubt. Neither would it be consistent with our experimental and computational observations that for aqueous

nucleobases, nucleosides, and nucleotides, changes in VIEs are in fact very small and, moreover, hydration leads to lowering rather than raising the VIE.[6, 7]

In this study, we establish the effect of the DNA environment on the VIE of an aqueous nucleobase by means of *ab initio* calculations supported by PES experiments. As a computationally tractable model, we took the widely studied Dickerson dodecamer as a small, but stable piece of DNA.[14] Out of the four bases, we focus here on guanine which has the lowest ionization energy and acts, therefore, as a sink for a migrating hole in DNA.[15] We can also infer, *per analogiam*, conclusions for the other nucleobases, which all have VIEs higher by about 0.5 eV.[6, 7] To attempt an experimental verification, we applied liquid jet PES to explore the lowest ionization energy region of genomic double-stranded DNA as compared to the component nucleotides. Both systems were investigated in buffered aqueous solution.

The computational setup was built upon our previous experience, which showed that for VIEs of nucleobases, nucleosides, and nucleotides the polarization effect of the aqueous medium can be quantitatively (with absolute accuracy better than ~0.5 eV) accounted for via a non-equilibrium polarizable continuum model.[6, 7] The selected guanine base of the Dickerson dodecamer, was treated at a correlated *ab initio* level (either alone (Figure 1A), or together with its neighboring stacked (Figure 1B) or hydrogen bonded (Figure 1C) nucleobase), while the remainder of the dodecamer was described at a semi-empirical level, as schematically depicted in Figure 1 (for more computational details see Supporting Information).

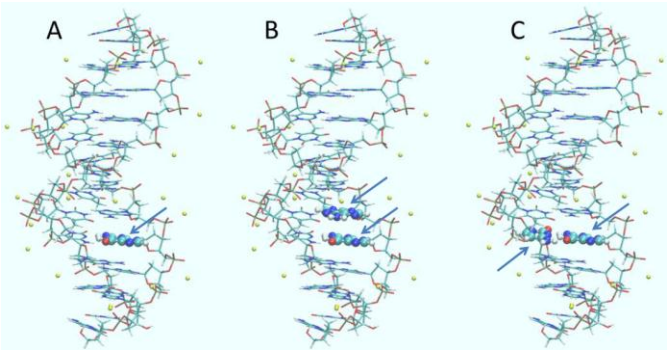

Figure 1. *Aqueous Dickerson dodecamer neutralized by sodium cations. Residues treated at the ab initioMP2/aug-cc-pvdz level of theory are represented by balls and sticks and highlighted by blue*

arrows: A) guanine, B) a stacked guanine-adenine pair, and C) a hydrogen bonded guanine-cytosine pair. The remaining atoms and ions were treated at the semi-empirical PM6 level. The light blue background indicates water modeled by a polarizable continuum model.

The results of the present calculations, together with our previous VIE values for canonical tautomers of aqueous guanine, guanosine, and guanosine monophosphate anion (GMP^-, low lying conformations)[7] are summarized in Table 1. Our method thus predicts the VIE of guanine in aqueous DNA to be around 7 eV with uncertainty of few tenths of an electronvolt, primarily due to residual inaccuracies in accounting for the aqueous[6, 7] and DNA[16] environments, likely leading to a small underestimation of the ionization energy. Strikingly, this value practically coincides with the calculated VIEs for the corresponding aqueous nucleotide, nucleoside, and nucleobase, which are only marginally higher (Table 1). In other words, the aqueous solvent with its extremely high dielectric constant is taking care of essentially all environmental effects on the VIEs of nucleobases. In DNA, this involves both direct screening the electronic influence of the fully hydrated charged phosphate groups and a longer range electronic screening of the less water exposed nucleobases. The solvent screening effect is not perfect, nevertheless, it reduces gas phase environmental changes in nucleobase VIEs of several electronvolts[6, 7] to a fraction of an eV in water (see Table 1 below and Table S1 in Supporting Information).

Table 1*. Calculated vertical ionization energies (in eV) of guanine in different arrangements in water.*

Guanine in DNA	7.1
Guanine in DNA (GC pair)	6.9
Guanine in DNA (GA stack)	7.0
Guanine in GMP^-	7.0 – 7.3[a]
Guanine in guanosine	7.4[a]
Guanine	7.3[a]

[a]Ref. 7.

Within the present calculations, the electrostatic and electronic polarization effects of the DNA, counterions, and water on the VIE of the guanine nucleobase were accounted for using the semiempirical PM6 method (DNA and counterions) and the non-equilibrium polarizable continuum approach (water). In order to demonstrate the importance of such a polarizable embedding we performed additional calculations, where electronic polarization of the DNA and aqueous environments were neglected, similarly as in Refs. 11 and 12. Two types of calculations were performed: The effect of the rest of the dodecamer with sodium counterions on the guanine nucleobase was either included within a point charge representation (electrostatic embedding) or it was only sterically constraining with all point charges set to zero (mechanical embedding) for the VIE calculation. In both cases, increasing amounts of explicit water molecules surrounding the dodecamer were represented as point charges within electrostatic embedding (for more details see Supporting Information). The results presented in Figure 2 show that the VIE converges when the radius of the water shell around the dodecamer becomes larger than ~10 Å, consistent with previous calculations.[12] However, even for the full electrostatic embedding in DNA and aqueous solution (black curve in Figure 2) the converged value overshoots the VIE of guanine from Table 1 by about 1.5 eV, which demonstrates the importance of electronic polarization effects of the environment,[6, 7] missing within this approach. Further neglecting the electrostatic effects of the DNA environment leads to exaggerated VIE values converging to almost 13 eV for a sufficiently large water shell (blue curve in Figure 2). This curve is remarkably similar to that presented in Figure 4 of Ref. 12, indicating that the previously reported VIEs in aqueous DNA environment are likely to be strongly overestimated due to an interaction imbalance caused by polarized water molecules next to the dodecamer, but turned off DNA and sodium charges. Such a conclusion is further supported by the fact that the other previous QM/MM study predicted only a modest effect (of less than 0.7 eV) of the electrostatic embedding of the aqueous DNA environment on the gas phase VIE of a nucleobase.[11] Note that this is consistent with the present electrostatic embedding (black curve in Figure 2) converging to a

value close to our previously calculated gas phase VIE of canonical guanine of 8.4 eV.[7] At the same time, we should stress that polarizable embedding shifts the VIE of guanine in aqueous DNA more than 1 eV below the corresponding gas phase value (Table 1 and red arrow in Figure 2).

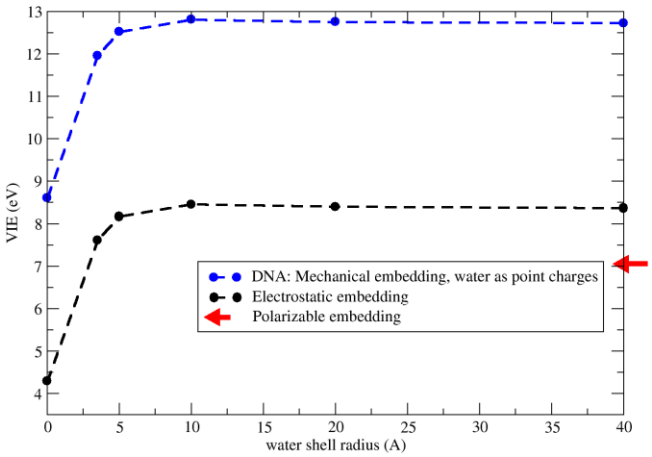

Figure 2. *Evolution of the VIE of guanine in the Dickerson dodecamer upon increasing the radius of the water shell included within electrostatic embedding. The DNA environment is included either via mechanical embedding (blue) or electrostatic embedding (black). The red arrow points to the value obtained from polarizable embedding designed in the present study.*

The present calculations were complemented by the first energy-resolved photoelectron spectroscopy experiments to be performed on DNA under aqueous buffered conditions. Because of the gram-scale requirements of the current liquid jet technique, sequence-specific samples are cost-prohibitive and so DNA solutions were prepared from sheared DNA extracted and purified from herring sperm (Sigma-Aldrich). The producer reports crude oligomers having length of 50 base pairs or less, nevertheless, characterization using gel electrophoresis employing molecular weight markers indicated presence of longer oligomers. Further UV-VIS spectroscopic characterization established a A_{260}/A_{280} ratio of 1.86, i.e., above the standard purity ratio of 1.8.[17] All PES measurements were performed at the BESSY synchrotron facility in Berlin under conditions analogous to our previous study of pyrimidine nucleosides (for experimental details see Supporting Information).[6] A comparison of photoelectron spectra of the aqueous genomic DNA vs. an equimolar mixture of nucleotides is presented in Figure 3. The spectra are

essentially identical at ionization energies below ~8.5 eV indicating that the DNA environment does not affect the lowest VIE. This spectral tail, extending to ~7 eV, corresponds to the VIEs of all the individual nucleobases in the DNA (red curve in Figure 3) or in the nucleotides (black curve in Figure 3). In fact, not only are the tails practically identical in shape, but also the very similar photoelectron count rates recorded from equivalent optical density solutions reinforces the idea that photoelectron bands of the nucleobase are not shifting within the featureless band shape. We note that at higher ionization energies, between 8.7 and 9.5 eV, contributions from (deoxy)ribose sugars and phosphate become significant.[6, 7, 18] In this region, the nucleotide mixture exhibits a shoulder at lower binding energies and the DNA sample at higher binding energies. Both can be attributed to contributions from the different charge states of the phosphate moiety present in the respective systems around pH 8.[18]

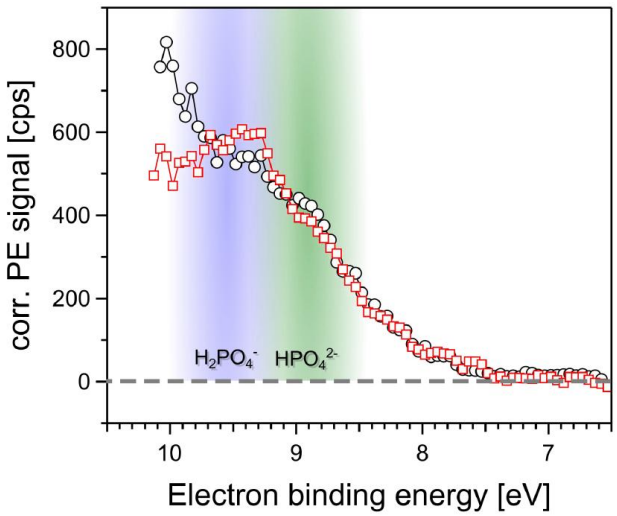

Figure 3. *Valence photoelectron spectra of sheared herring sperm DNA (pH 7.7, red squares) and a solution that contains an equimolar mixture of all four nucleotides (pH 8, black circles). Solutions have identical OD_{260} and experiments were run back to back such that the photoelectron counts per second (cps) can be compared (see SI for details). Photoelectron band positions for different labeled charge states of inorganic phosphate are shown by blue and green shading (from Ref. 18).*

In summary, the present computational and experimental study clearly demonstrates that the surrounding DNA has only a negligible effect on ionization energies of nucleobases in their native aqueous environment. This is due to the remarkable ability of the aqueous solution to screen the electronic effects of the sugar-phosphate backbone and the neighboring nucleobases on the VIEs. The present result thus grossly simplifies considerations concerning ionization processes in aqueous DNA by

the fact that reliably established vertical ionization energies of nucleobases, nucleosides, or nucleotides in water can be directly employed in studies of DNA in its native environment.

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