

Ionization Energies of Aqueous Nucleic Acids: Photoelectron Spectroscopy of Pyrimidine Nucleosides and *ab Initio* Calculations

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Abstract: Vertical ionization energies of the nucleosides cytidine and deoxythymidine in water, the lowest ones amounting in both cases to 8.3 eV, are obtained from photoelectron spectroscopy measurements in aqueous microjets. *Ab initio* calculations employing a nonequilibrium polarizable continuum model quantitatively reproduce the experimental spectra and provide molecular interpretation of the individual peaks of the photoelectron spectrum, showing also that lowest ionization originates from the base. Comparison of calculated vertical ionization potentials of pyrimidine bases, nucleosides, and nucleotides in water and in the gas phase underlines the dramatic effect of bulk hydration on the electronic structure. In the gas phase, the presence of sugar and, in particular, of phosphate has a strong effect on the energetics of ionization of the base. Upon bulk hydration, the ionization potential of the base in contrast becomes rather insensitive to the presence of the sugar and phosphate, which indicates a remarkable screening ability of the aqueous solvent. Accurate aqueous-phase vertical ionization potentials provide a significant improvement to the corrected gas-phase values used in the literature and represent important information in assessing the threshold energies for photooxidation and oxidation free energies of solvent-exposed DNA components. Likewise, such energetic data should allow improved assessment of delocalization and charge-hopping mechanisms in DNA ionized by radiation.

Introduction

One of the principal effects on cells of high-energy radiation such as that encountered in radiation therapy or from cosmic rays is the ionization of DNA.¹ Similarly, high-energy photons can directly ionize a base within DNA; in both cases the impact of the radiation is to lead to an oxidized center or “hole”. While any DNA component can be initially ionized, typically, the hole ends up localized on a guanine base as it bears the lowest *in*

situ oxidation potential.² The early processes leading to such radiation-induced damage are not well understood.^{3–6} Surprisingly, this includes primary experimental information on the ionization energetics of each of the four constituent bases of aqueous DNA.^{7,8} Moreover, in double-stranded DNA, the sugar

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phosphate backbone is the most exposed to the solvent. Direct ionization, as well as oxidative damage by hydroxyl radicals also produced by ionizing radiation,¹ can occur at this locus and eventually leads to strand breaks.⁹ Therefore, the ionization energies from the molecular orbitals not only on the bases but also on the sugar and phosphate backbone components of nucleotides are relevant for unraveling mechanisms of DNA damage.

In order to understand charge mobility along the double helix, determinations of both the vertical and adiabatic (or threshold) ionization potentials (IPs) of each of the bases within realistic environments are required. Attention has focused on establishing the ionization threshold in solution and whether direct one-photon oxidation can occur at the edge of the solar UV-B region. Deoxyguanosine, which contains the most readily ionized base, guanine, is the only system with a clear-cut measurement of the ionization threshold. Using low-intensity illumination and electron scavenging, LeBreton and co-workers established a threshold at ~ 4.8 eV.¹⁰ In laser excitation experiments, because the electron and ionization product yields are very low, high-intensity excitation is needed, thus requiring difficult separation of one- versus two-photon processes.^{12,13} Most thresholds currently cited actually come from semiempirical estimates⁸ based on gas-phase vertical ionization potentials (VIPs) and the application of large thermodynamic corrections. Alternatively, calculations starting with energy changes in vacuum and then employing various reaction field and empirical corrections for the hydration of products have been published.⁷ Direct measurement of the solution VIP as presented here can be expected to substantially improve threshold energy estimates for DNA bases.

Early gas-phase photoelectron spectra of the bases studied by Hush and Cheung,¹⁴ as well as of a range of base^{15–17} and nucleoside¹⁸ analogues, have been reported by LeBreton and co-workers and Kubota and Kobayashi.¹⁹ The most relevant to the current study are those reported by Yu et al. for thymine and cytosine bases and *O*-methyl derivatives of deoxythymidine and cytidine nucleosides.¹⁸ There have been several more recent measurements exploring the effect of microhydration on the ionization energy of bases in gaseous molecular beams. Rather than the photoelectron spectrum, which reports on electron removal from each of the highest-lying molecular orbitals, these experiments only provide the appearance threshold for observing

cations in a mass spectrometer.^{20–22} Recently, Wang and co-workers have succeeded in getting all four deoxynucleotides (dNMPs) into the gas phase and recording their photoelectron spectra.²³ Their supporting hybrid density functional calculations indicate that, for dNMPs in vacuum, the lowest IP arises for removing an electron from the negatively charged phosphate, except for dGMP[−], where the base orbital is the lowest.²³ Using a partial third-order self-energy approximation method, Ortiz et al. concluded that states originating from ionization of phosphate and the base are almost degenerate for dAMP[−] and dTMP[−], while the lowest ionization of dCMP[−] is phosphate centered.²⁴ Serrano-Andres and co-workers then demonstrated that at the CASPT2 level the lowest ionization of dTMP[−] comes from the base, with the state originating from phosphate ionization lying only 0.2 eV higher.²⁵ Additionally, these computational studies show for gas-phase pyrimidine systems that the base orbital energies change on substitution of the aromatic ring, e.g., on addition of the sugar moiety, on addition of the unscreened charge of the phosphate group, or on microhydration around the base.

The effects of bulk hydration and of counterions are expected to lead to large changes, possibly reordering the orbital energies. This might be expected to be most dramatic for the VIPs of orbitals on the negatively charged phosphate. However, there should also be changes in the orbital energies of the base (and sugar) components connected with the longer-range polarization of the water solvent or due to π -stacking of bases in double-stranded DNA. Here we explore the former effect, i.e., that of the full aqueous environment, on ionization energetics. This is done via a combination of *liquid phase* photoelectron spectroscopy of two nucleosides and electronic structure calculations of DNA components that explore a nonequilibrium polarizable continuum model (NEPCM)^{26–29} for the water environment. These are the first reports of experimental solution-phase IPs of the pyrimidine DNA building blocks, and we can use the experiment to appraise the accuracy of the various theoretical solvation models on this important biochemical system.

Ab initio electronic structure calculations have been extensively applied for ionization of DNA components in the gas phase^{24,25,30–32} and in microhydrated environments containing a small number of water molecules.^{33,34} Extensions toward bulk

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solvation employ a standard (equilibrium) polarizable continuum model (PCM) of the solvent which, however, corresponds not to vertical but to adiabatic ionization of the solute.^{35,36} Alternatively, a thermodynamic cycle, which is also pertinent to an adiabatic ionization process, is used.⁸ Very recently, QM/MM methods have been applied to obtain vertical ionization energies of cytosine with water and counterions included from molecular dynamics simulations.³⁷ Here, we show that *ab initio* electronic structure calculations employing a nonequilibrium version of a PCM can efficiently and with sufficient accuracy account for bulk solvent effects on ionization of DNA building blocks. Such calculations not only quantitatively reproduce the experimental VIPs but also allow for a detailed interpretation of the observed photoelectron spectra in terms of the underlying electronic states.

Methods and Materials

Experimental Details. Photoemission (PE) measurements from aqueous solutions of cytidine (C₉H₁₃N₃O₅) and of deoxythymidine (C₁₀H₁₄N₂O₅) were performed at the U41 PGM undulator beamline of the synchrotron radiation facility BESSY, Berlin. PE spectra were collected from a 15 μm liquid microjet traveling at a velocity of 120 m/s with a temperature of 4 °C. In the present valence photoelectron study we have used a photon energy of 200 eV. Details of the experimental setup have been described previously.^{38,39} Briefly, excitation is carried out with the synchrotron light polarization vector parallel to the flow of the liquid microjet, while the mean electron detection angle is normal to the polarization vector. Photoelectrons are collected through an orifice 200 μm in diameter, which acts as a conductance-limiting aperture to differentially pump the main chamber housing the liquid microjet (operating at 10⁻⁵ mbar) from the hemispherical energy analyzer (operating at 10⁻⁹ mbar). Energy resolution of the beamline was better than 200 meV at the incident photon energy used here. The resolution of the hemispherical electron-energy analyzer is constant with kinetic energy (200 meV at a constant analyzer energy of 20 eV). Typical signal count rates were 10³–10⁴ s⁻¹. The small focal size (23 × 12 μm²) of the incident photon beam at the U41 PGM beamline allows for matching spatial overlap with the liquid microjet, which limits the contribution of gas-phase components in the collected PE spectra to <5% for liquid water. Electron kinetic energies are calibrated on the basis of the 1b₁ binding energy of liquid water.⁴⁰

Highly demineralized water was used for preparing the 0.2 M cytidine and deoxythymidine aqueous solutions. The purity of both chemicals was >99% (Sigma-Aldrich). The pH values of the as-prepared solutions were 7.56 and 7.05, respectively.

Computational Details. The ground-state geometries of all investigated systems (i.e., CMP⁻, dTMP⁻, CMP²⁻, dTMP²⁻, cytidine, deoxythymidine, cytosine, thymine, ribose, deoxyribose, and dihydrogenphosphate anion) were optimized in the gas phase at the MP2/aug-cc-pVDZ level. We have considered only canonical forms of both cytosine and thymine molecules. Canonical tautomers dominate in water environment for both molecules, and the canonical form is also the most stable structure for thymine in the gas phase.^{41,42} (For cytosine, one of the enol forms is more stable in the gas phase; however, as few as two solvating water molecules

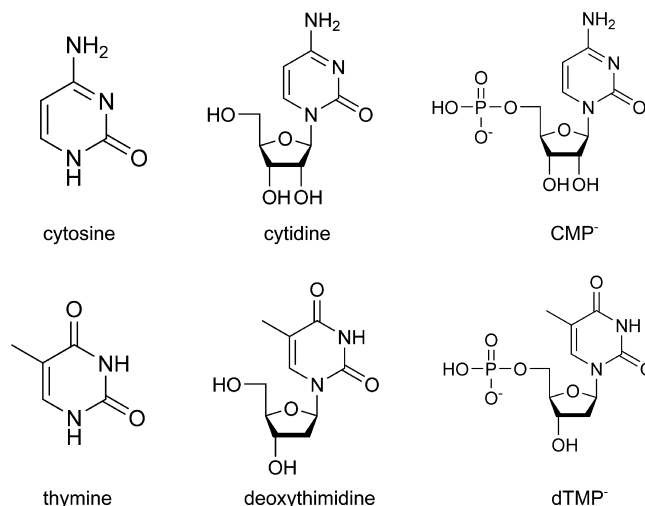


Figure 1. Standard representation of the chemical structures of the pyrimidine bases, nucleosides, and nucleotides.

change the energy ordering in favor of the canonical form.⁴¹) The chemical structures of the pyrimidine bases, nucleosides, and nucleotides are presented in Figure 1. At optimized geometries, VIPs were evaluated. To calculate the lowest IP, we used the unrestricted version of the MP2 method. Higher spin components were annihilated via Schlegel's projection method (PMP2). The PMP2 approach was demonstrated to provide excellent values of ionization energies for isolated gas-phase nucleic acid bases.⁷

To evaluate higher IPs, i.e., to simulate the PE from orbitals energetically below the highest occupied molecular orbital (HOMO), we utilized the time-dependent version of the density functional theory (TDDFT). The *n*th IP was then calculated as the sum of the first ionization potential, IP₁ (calculated at PMP2/aug-cc-pVDZ level), and the (*n* - 1)th excitation energy of the ionized species (calculated at TDDFT/6-31g* level). This level of theory allows for a combination with NEPCM to account for solvent effects (*vide infra*). Photoionization probabilities are considered to be the same for all these ionized states, which is a reasonable approximation for high-energy photons used in the experiment.

Note that density functional methods suffer less from spin contamination than the MP2 method; however, they can predict spurious charge-transfer states due to the self-interaction error.⁴³ This issue is particularly relevant for larger species such as CMP⁻ or dTMP⁻. Functionals with a low fraction of exact exchange provide low IPs for states with a charge transfer between base, sugar, and phosphate moieties. This can be rectified by employing hybrid functionals with a large portion of exact exchange, such as the new and accurate BMK functional.⁴⁴ Table 1 shows benchmark calculations for the lowest four IPs of gas-phase cytosine, thymine. We see that the TDDFT method with the BMK functional performs very well compared to experiment, being of comparable accuracy to the computationally much more demanding CASPT2 method. Note that other hybrid functionals, B3LYP and BHandHLYP, proved to be somewhat less accurate.

The effect of the aqueous solvent on ionization was accounted for using the PCM.^{45–47} The standard PCM procedure applied both before and after ionization corresponds to adiabatic IPs. Since our

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Table 1. Lowest Four Ionization Energies (in eV) of Gas-Phase Pyrimidine Bases^a

	MP2 + BMK	CASPT2 ^b	expt ^c
cytosine	8.84	8.73	8.82
	9.38	9.42	9.45
	9.66	9.49	—
	10.00	9.88	9.90
thymine	9.15	9.07	9.20
	10.09	9.81	10.05
	10.45	10.27	10.44
	10.92	10.49	10.88

^a This table provides benchmarking of the applied method against CASPT2 calculations and experiment. ^b Reference 30. ^c References 17, 53, 54.

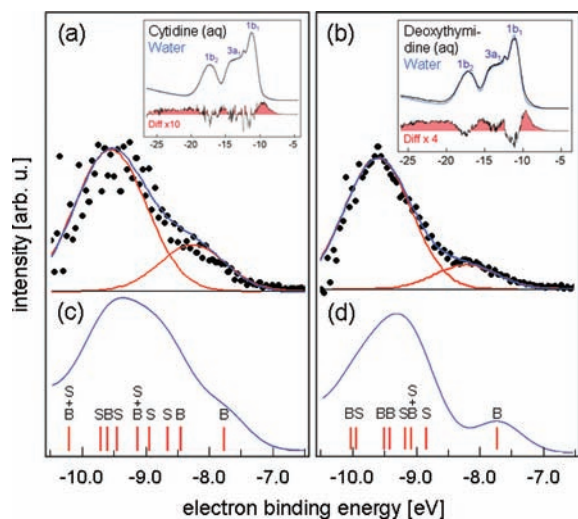


Figure 2. Experimental and computed photoelectron spectra of cytidine and deoxythymidine. Insets: Experimental spectra of (a) 0.2 *m* cytidine and (b) 0.2 *m* deoxythymidine aqueous solution (black curves) and of neat liquid water (blue curve), both measured at photon energy of 200 eV and collected over 10 individual runs. The difference spectrum is shown at the bottom; the positive signal (in red) arises from solute contributions. Photoemission from the three water valence orbitals, 1b₂, 3a₁, and 1b₁, is labeled. The main panels in (a) and (b) show increased detail of the difference spectra including two-Gaussian fits with lowest ionization peak at 8.3 eV for both species. Computed photoelectron spectra of aqueous cytidine (c) and deoxythymidine (d) (red bars), broadened by Gaussians with fwhm of 0.9 eV (thick blue line). The origin of each ionization transition is labeled as B for base or S for sugar.

principal aim was to calculate vertical IPs, we optimized the reaction field only for the parent species before ionization. In a subsequent step we have used the NEPCM protocol as implemented in the Gaussian 03 code.^{26,27,48} In this way, only the fast electronic component of the solvent response to ionization is accounted for, and the slow component of this response (corresponding to nuclear relaxation) is left out. The NEPCM approach combined with the PMP2 method was thoroughly tested for aqueous anions, neutral molecules, and cations in our previous studies.^{28,29}

Results

Valence photoelectron spectra of aqueous cytidine and deoxythymidine, measured at 200 eV photon energy, are presented in Figure 2a,b. For comparison, in the inset the PE spectrum of pure water is also depicted, the effect of the added solutes being highlighted by the differential spectra shown at the bottom of each inset and (in more detail) in the main panel

of the graphs. Before subtraction, the spectra are normalized at the background intensity around -25 eV BE. The most interesting signature of cytidine or deoxythymidine is the relatively broad spectral feature between roughly -6.5 and -10.5 eV, i.e., before the rise of the first water peak (1b₁). Photoelectron intensities from these solutes obtained in the experiment are relatively high, even though the concentrations were only 0.2 *m* (which corresponds to almost maximum solubility). Note that, for alkali halide aqueous solutions at the same low concentration, solute signal is barely detectable. Since for the photon energy used here PE spectroscopy is surface selective,³⁸ this behavior reflects a considerable surface activity of cytidine and deoxythymidine (being larger for the latter case). For both species, the spectral signature consists of a lower intensity region at smaller binding energies and a more intense region at higher energies. Both of these features are broad, and they overlap to some extent. They can be fitted by two Gaussians, centered at -8.3 and -9.6 eV for cytidine, and -8.3 and -9.5 eV for deoxythymidine (Figure 2a,b). However, since the features are broad and overlapping, they are difficult to assign without the help of electronic structure calculations which provide further analysis and interpretation of the underlying states.

The calculated spectra for aqueous cytidine and deoxythymidine in the low electron binding energy region, corresponding to the part of the experimental signal not obscured by water features, are shown in Figure 2c,d. Vertical bars depict the individual vertical ionization transitions of aqueous cytidine and deoxythymidine, evaluated within the NEPCM (the lowest ionized state being evaluated at the PMP2 level, while the TDDFT method is employed for the higher states). The letter assignment above each bar indicates whether the electron is ejected from an orbital dominantly situated at the base (B) or sugar (S). The spectral envelope in Figure 2c,d is obtained by broadening the calculated discrete spectra using Gaussians of full width at half-maximum (fwhm) 0.9 eV, which corresponds to a typical vibrational and inhomogeneous broadening in polyatomic aqueous solute systems.⁴⁸ We have assigned equal intensities to all lines, a reasonable assumption born out by the agreement with experiment showing that, with incident photon energies well above threshold, the photoionization cross sections do not vary dramatically between states.

We see from Figure 2 that the low-energy feature is indeed composed from a single peak, as suggested by the fit to the experimental spectra. This peak reflects the formation of the ground electronic state of the cation of cytidine or deoxythymidine, with the solute and solvent geometry corresponding to the neutral species as pertinent to vertical ionization. The calculated values of the lowest electron binding energy are -7.78 eV for cytidine and -7.74 eV for deoxythymidine. For both aqueous cytidine and deoxythymidine, this lowest ionization takes place at the pyrimidine base. This is demonstrated in Figure 3a,b, which shows the HOMOs of the two species in water prior to ionization, localized at the respective base. Qualitatively the same picture is also seen in the unpaired spin density after ionization, which is depicted for both species in Figure 3c,d. We note here that, upon adding the phosphate group, the lowest ionized state remains localized on the base, a point relevant to later discussion.

The agreement between the theoretical and experimental spectra over the range reported is excellent (Figure 2). The calculations are correctly reproducing the two overlapping spectral features, including their relative intensities. Qualita-

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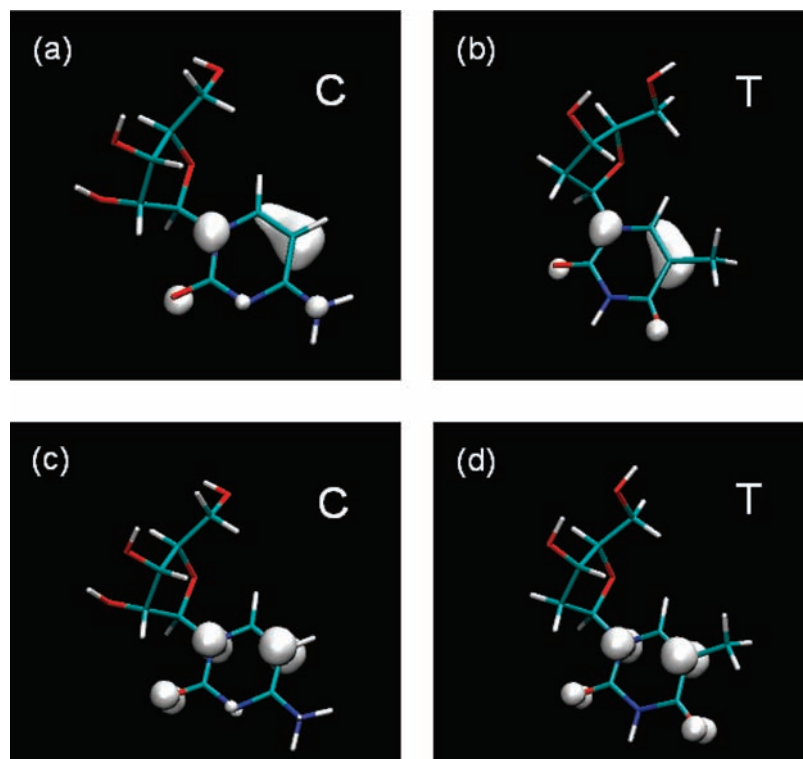


Figure 3. Highest occupied molecular orbitals of (a) cytidine and (b) deoxythymidine in water prior to ionization and the spin densities of (c) cytidine and (d) deoxythymidine cations in water after ionization.

tively, there is little difference between the photoelectron spectra of cytidine and deoxythymidine, whereas quantitatively the low-energy peak is slightly more pronounced and the higher energy peak broader and lower for the latter species. A great asset of the calculations is that they allow for interpretation of the observed spectral features in terms of electronic states localized on different parts of the solute. To this end, we have modeled (in addition to aqueous cytidine and deoxythymidine) also the photoelectron spectra of aqueous cytosine, thymine, ribose, deoxyribose, and dihydrogenphosphate anion, together with CMP^- and dTMP^- (Figure 4). The picture emerging from these calculations is as follows. To a good approximation, the aqueous spectrum of CMP^- or dTMP^- can be decomposed into additive contributions from their building blocks, which indicates a relatively weak electronic interaction between them in water. The weakest binding energy feature originates solely from vertical ionization of the base (cytidine or deoxythymidine). In contrast, the higher binding energy feature is composed from states localized on the base, sugar, and phosphate. This region is, therefore, characterized by a mixture of states from all constituent parts of the solutes.

The lowest IPs of all aqueous species considered so far and additional data for 1-methylthymine, 1-methylcytosine, and the nucleotide dianions are summarized in Table 2. Note both mono- and dianionic forms of nucleotides (albeit with transient counterion binding) are present at neutral pH; therefore, both were considered. The table presents vertical values obtained using NEPCM, together with equilibrium PCM. These are two very different quantities, the former best representing vertical ionization with only fast electronic relaxation of the solvent and the latter adiabatic ionization, which additionally models the slow solvent nuclear relaxation. We see that solvent nuclear relaxation is connected with a sizable decrease of about 1.1 eV of the IPs of all species containing the pyrimidine bases. For

the sugars and dihydrogen phosphate this reduction is even larger, 0.9–1.4 eV in the former case and 1.3 eV in the latter. Further relaxation of the solute intramolecular framework in systems containing pyrimidine base and sugars is less important, leading to an additional decrease of the IPs of less than 0.3 eV. However, it is more important to relax intramolecular modes for H_2PO_4^- , leading to an additional decrease of the IP of more than 0.9 eV. We note that in water the VIPs of ribose, deoxyribose, and dihydrogen phosphate are more than 1.2 eV higher than those of the bases. Even in the adiabatic framework this gap persists, albeit being slightly reduced to ~ 1 eV.

Table 2 underlines a rather unexpected result: there is virtually no change (i.e., <0.15 eV) in the vertical or adiabatic IPs of aqueous pyrimidine bases upon substitution at the 1- position, either by adding a methyl or sugar group, or by adding a sugar–phosphate group. Moreover, in water the difference in IPs of nucleotides with a monobasic versus dibasic phosphate group is also below 0.15 eV. This stability of IPs is in stark contrast to the situation in the gas phase, as shown for the lowest IPs in Table 3. The difference between aqueous and gas-phase environments can be also easily seen upon comparing the calculated spectra in Figure 4a,b with those in Figure 4c,d. Note that in order to directly compare aqueous with vacuum systems we applied the same 0.9 eV broadening, although actual experimental gas-phase peak widths are substantially narrower.⁸ Upon dehydration, electron binding energies of CMP^- and dTMP^- decrease by 2 eV, primarily due to destabilization of the parent anion. More strikingly, the spectrum of CMP^- or dTMP^- in a vacuum can no longer be viewed as a sum of its constituent parts, of which the smallest IP in the gas phase is that of H_2PO_4^- , while all the other species exhibit electron binding energies larger by several electronvolts (Table 3). Analysis of the molecular orbitals of the parent species in the gas phase (figures not shown here) reveals that for CMP^- and

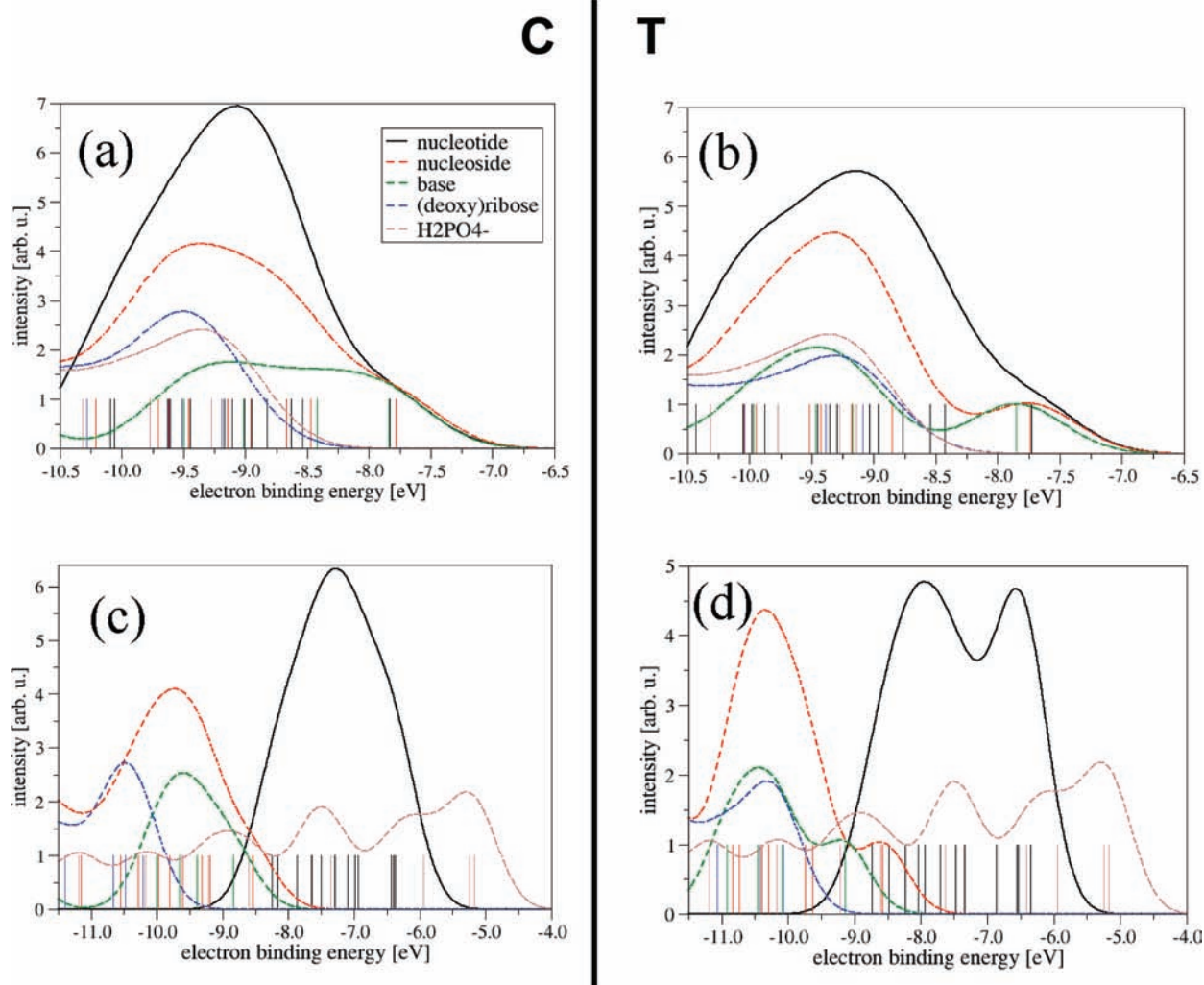


Figure 4. Comparison of simulated photoelectron spectra in liquid water and gas phase. Computed spectra of (a,c) CMP^- , cytosine, cytosine, ribose, and the dihydrogen phosphate anion and (b,d) dTMP^- , deoxythymidine, thymine, deoxyribose, and the dihydrogen phosphate anion. Computed spectra in water are shown in (a,b), while those in the gas phase are shown in (c,d). All stick spectra were broadened by Gaussians with fwhm of 0.95 eV.

Table 2. Calculated Vertical (NEPCM) and Adiabatic (PCM and PCM with Relaxed Solute) Ionization Energies of Aqueous Pyrimidine DNA Components Together with Current Experimental Values for Aqueous Pyrimidine Nucleosides (in eV)

	PMP2 NEPCM	PMP2 PCM	PMP2 PCM with relaxed solute
cytosine	7.86	6.59	6.34
1-methylcytosine	7.75	6.60	6.36
cytidine	exp. 8.3	7.77	6.64
CMP^-	7.83	6.72	6.12
CMP^{2-}	7.70	6.63	6.04
thymine	7.87	6.65	6.39
1-methylthymine	7.75	6.62	6.41
deoxythymidine	exp. 8.3	7.76	6.66
dTMP^-	7.70	6.67	6.14
dTMP^{2-}	7.68	6.62	6.08
ribose	8.72	7.85	7.32
deoxyribose	9.10	7.75	7.43
H_2PO_4^-	8.94	7.61	6.68

dTMP^- the lowest ionization still comes from the base, while for gaseous CMP^{2-} and dTMP^{2-} it comes from the phosphate with a dramatically reduced IP due to the presence of the additional electron (Table 3). Returning to gas-phase CMP^- or dTMP^- , the base-centered HOMO is strongly destabilized by

Table 3. Calculated and Experimental Gas-Phase Vertical Ionization Energies of Pyrimidine DNA Components (in eV)

	PMP2	experiment
cytosine	8.84	8.82 ^a
1-methylcytosine	8.79	8.65 ^b
cytidine	8.56	8.46 ^c
CMP^-	6.37	5.80 ± 0.5 ^d
CMP^{2-}	1.62	
thymine	9.15	9.20 ^c
1-methylthymine	8.74	8.79 ^b
deoxythymidine	8.58	
dTMP^-	6.35	5.85 ± 0.5 ^d
dTMP^{2-}	1.54	
ribose	10.21	
deoxyribose	10.07	
H_2PO_4^-	5.83	5.06 ^a

^a Reference 53. ^b Reference 8. ^c Reference 18. Experimental data are for a cytidine analogue (2',3',5'-tri-*O*-methylcytidine) that can be volatilized. Substitution on the ribose ring is not expected to shift the lowest IP. ^d Reference 23. ^e References 17 and 54.

the presence of the phosphate. The next IP, which is almost degenerate with the lowest one, then originates from the phosphate group.

The monobasic phosphate group is responsible for lowering the gas-phase IP of the pyrimidine by 2 eV, and that of the dibasic group by a further 4.5 eV. Compared to this, the influence of the sugar is more modest. Nevertheless, it still leads to a 0.3–0.5 eV shift toward smaller electron binding energies in a vacuum (Table 3). Unlike those in water, the gas-phase IPs of the pyrimidine bases thus change appreciably upon substitution at the 1-position on the pyrimidine ring. This effect is stronger for thymine than for cytosine. It is important to point out that calculated lowest IPs of the constituent parts are in excellent agreement with gas-phase experiments. This is also true for CMP^- and dTMP^- , where the present calculations fall within the large uncertainty of the experimental values (Table 3).

Discussion

The present experimental and computed photoelectron spectra for the aqueous pyrimidine nucleosides are in very good agreement with each other. In particular, calculations are capable of faithfully modeling the shape of the experimental spectra. Taking this together with the excellent performance of the theoretical models employed in reproducing gas-phase VIPs³¹ including the lowest states of the base and phosphate moieties, we have established a reliable and computationally inexpensive approach for evaluating and interpreting photoionization experiments on aqueous DNA components. Note, however, that the values of VIPs of aqueous cytidine and deoxythymidine based on NEPCM calculations are about 0.5 eV smaller than those obtained from the two-Gaussian fit to the experimental photoelectron spectra (Table 2 and Figure 2). The calculations somewhat underestimate the IPs due to the neglect of the detailed molecular structure of the first solvent shell around the solutes within the polarizable continuum solvent model. Indeed, our previous calculations of aqueous imidazole²⁸ showed that explicit inclusion of up to five water molecules in the immediate solvation shell into the NEPCM calculation leads to an increase of the VIP by 0.2 eV, with the effect that more distant explicit solvent molecules are estimated to account for an additional 0.2 eV. Extrapolating analogous corrections also for aqueous cytidine and deoxythymidine brings the calculation within 0.1 eV of the fit to the experiment.

On the experimental side, as commented earlier, the recorded nucleosides signals are stronger than those for inorganic ions at the same concentration, presumably due to enhanced surface activity. The photoelectron spectroscopy experiment preferentially probes the interfacial region,³⁸ particularly for the photon energies employed here. This is due to the fact that the electron inelastic mean free path in water is short, amounting to only several water layers for the present kinetic energies of the photoelectrons.³⁸ Some of the discrepancy between theory and experiment may thus also come from differences between interfacial and bulk solvation, the former involving an incomplete solvation sphere. However, our previous study of aqueous iodide shows that the difference between IPs of bulk and interfacial ions is only of the order of 0.1 eV.⁵⁰ An additional subtlety results from the way the experimental spectra are fitted to a reduced set of two Gaussians. Although justified by the level of detail resolved in the experiment, if a set of Gaussians corresponding to the actual number of the calculated states were

used, the lowest fitted Gaussian would be narrower and shifted toward the calculated value. The inherent vibrational and inhomogeneous broadening of the experimental spectra highlights the point that a detailed analysis of the electronic states of the solutes can only be achieved with an underlying theoretical model. Calculations clearly interpret the higher binding energy part of the spectrum as originating from a multitude of electronically excited states of the nascent cation, while the low binding energy part is due to a single (ground) state localized on the base.

For comparison, the vertical ionization energies of uracil as well as the RNA polynucleotides (poly rU and poly rC) in cryogenic thin films on a pyrolytic graphite substrate have been measured recently. These reveal a binding energy of 8.05 eV for uracil, 8.1 eV for cytidine in poly rC, and 8.5 eV for uridine in poly rU.¹¹ These results are in qualitative agreement with those presented here, although the immediate environment of the base clearly differs. This good agreement can also be partly attributed to the relatively small change of the lowest IP of cytidine and deoxythymidine upon moving from the gas phase into water (compare Tables 2 and 3). Note that the solvent effect on the bare base is larger and comparable to that observed in our previous study on imidazole.²⁸ However, the sugar and phosphate moieties act as “presolvating” agents, thus reducing the effect of the aqueous solvent on the IP (*vide infra*).

Arguably the most dramatic result of the present NEPCM calculations is the prediction of a negligible (<0.15 eV) effect on the IPs of the aqueous bases of substitution on the base by adding a methyl, sugar, or sugar–phosphate group. This situation is in strong contrast with that in the gas phase, where the IP of the base is highly sensitive to both substitution and the local electrostatic environment. In terms of electronic structure, 1-methylcytosine and 1-methylthymine are better analogues to the nucleosides than the free bases, as they include the electronic effect of substitution at the 1-position on the pyrimidine ring. There is a marked decrease (~0.4 eV) in the gas-phase VIP particularly for thymine on methyl substitution at the 1-position nitrogen,⁸ which is reproduced well in our calculations. This shift is largely removed by the screening effect of the solvent, as is also the case on substitution with a sugar group at the 1-position. This screening is even more striking in the case of adding a charged phosphate group, which leads to lowering of the IP in the gas phase by 2–2.5 eV.⁵¹ Even deprotonating the phosphate group, so that it bears a double negative charge, has only a minor effect on the IP after dielectric screening by the aqueous solvent. This latter computational result is confirmed by our preliminary photoelectron spectra for aqueous CMP^{2-} which can be fit with two Gaussian peaks at 8.5 and 9.3 eV. These very modest shifts compared to aqueous cytidine are consistent with the spectral simulation shown in Figure 4 for CMP^- and our computed results for CMP^{2-} . The present calculations therefore highlight the limitations of gas phase data to provide a predictive capability for ionization of DNA components in bulk water. Further, these observations suggest a remarkable ability of bulk water to screen the electronic effects of components attached to the DNA bases.

It is worth stressing that standard (equilibrium) PCM calculations, which effectively relaxes the solvent after ionization, underestimate the present experimental IPs by more than 1.5

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eV (see Table 2). Such PCM calculations^{33–35} are adiabatic with respect to the solvent coordinates and thus cannot represent true vertical ionization. Therefore, standard PCM calculations should not be directly compared to experimental VIPs but rather to the ionization onsets. A similar argument also applies to estimates of IPs combining gas-phase calculations with hydration free energies.⁷ Experimental measurements for the ionization onsets of the free bases have been made for microhydrated clusters and compared with *ab initio* cluster calculations.^{33,36} Herschbach and co-workers estimate the adiabatic IP of thymine changing from 9.15 to 8.5 eV upon moving from a bare base to a cluster with three water molecules.²⁰ Belau et al. have observed somewhat smaller (~ 0.3 eV) shifts in ionization onsets for hydrated thymine and cytosine clusters of similar size.²² These shifts represent the solvent reorganization accompanying relaxation from the vertical water geometry to relaxed solvent structures around the cation. From the current experiments, we can establish only an upper bound for the ionization onset in bulk water (with respect to the vacuum level) at ~ 6.8 eV for both deoxythymidine and cytidine (Figure 2), which is at least 1.5 eV lower than that for the base(H_2O)₃ clusters. Essentially, increasing hydration on the pyrimidine nucleosides leads to a relatively small shift in the VIP but shifts the adiabatic onset to much lower energies because of the large solvent reorganization from multiple solvent shells.

The VIPs presented provide valuable information on the limiting energetics of the Rydberg manifold of electronically excited states of the closed-shell aqueous bases. The upper Rydberg-like states may dominate the excited-state absorption observed in femtosecond studies following the electronic relaxation of nucleobases.⁵² To evaluate charge-hopping mechanisms in oligomeric DNA, it will be interesting to see if the ionization energetics of bases change markedly when the bases are sequestered in the hydrophobic environment of the double helix.³⁷ Our present study serves as a possible prelude to direct measurements of ionization energetics within oligomeric seg-

ments of DNA of increasing length and within the native aqueous environment.

Conclusions

Photoelectron spectroscopy from an aqueous microjet has been used together with *ab initio* calculations employing a nonequilibrium polarizable continuum model for characterizing ionization of pyrimidine components of DNA in bulk water. The lowest vertical ionization energies of aqueous cytidine and deoxythymidine were determined experimentally as 8.3 eV for both species. Calculations, which are in quantitative agreement with the experiment, allow for a detailed interpretation of the photoelectron spectrum in terms of the underlying electronic states. A dramatic effect of the aqueous environment is also revealed by the *ab initio* computations. Namely, bulk water not only modestly lowers the ionization potential of the DNA bases but also makes it insensitive to the presence of sugar or phosphate. This is a very different situation from that observed in the gas phase, where the other DNA components (phosphate in particular) strongly influence the ionization process at the base. Further, the present results suggest that the lowest energy ionization process originates by electron ejection from the base, even in the aqueous pyrimidine nucleotides.

The present vertical ionization potentials and photoelectron band shapes for the aqueous pyrimidine DNA components provide important energetics data for modeling photoionization and charge-transfer processes in DNA. We therefore expect these results, along with future data employing the techniques reported here for purine and oligomeric DNA, to enable a better understanding of radiation-induced processes that lead to DNA damage in cells.

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