

Ionization of Aqueous Cations: Photoelectron Spectroscopy and *Ab Initio* Calculations of Protonated Imidazole

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Abstract

Photoelectron spectroscopy and *ab initio* calculations employing a non-equilibrium polarizable continuum model were employed for determining the vertical ionization potential of aqueous protonated imidazole. The experimental value of 8.96 eV is in an excellent agreement with calculations, which also perform quantitatively for ionization of aqueous alkali cations as benchmark species. The present results show that protonation of imidazole shifts up its vertical ionization potential in water by 0.7 eV, which is significantly larger than the resolution of the experiment or the accuracy of the calculation. Therefore, this combined experimental and computational approach can be applied for quantitatively analyzing the protonation state of histidine, of which imidazole is the titratable side chain group, in aqueous peptides and proteins.

1. Introduction

Aqueous cations represent a considerable challenge from the point of view of photoelectron (PE) spectroscopy at least for two reasons. First, until recently this high vacuum technique has not been applicable to volatile liquids such as water and aqueous solutions, except for concentrated systems close to saturation which have a significantly reduced vapor pressure.^{1,2} Only with the advent of the liquid microjet technique it became possible to record PE spectra of water and ions dissolved therein.^{3,4} Second, ionization of cations typically requires shorter wavelengths than those accessible via common laser sources. Synchrotron radiation, which allows accessing both valence and core ionization energies, has been recently used to study simple alkali cations,^{2,5,6} as well as molecular cations such as hydronium,⁷ tetrabutylammonium,^{8,9} or protonated amino acids.¹⁰

The above mentioned study of lysine demonstrated the ability of core electron PE spectroscopy to distinguish between different protonation states of an amino acid and to quantify their abundancies at varying pH.¹⁰ From this point of view, arguably the most interesting amino acid is histidine, which due to the value of $pK_a = 6$ of its imidazole side chain group changes protonation state around neutral pH. Recently, we have characterized the deprotonated (neutral) state of aqueous imidazole and its vertical ionization potential (VIP) by means of PE spectroscopy and *ab initio* calculations employing a non-equilibrium polarizable continuum solvent (NEPCM) model.¹¹ In the present study, we focus on the protonated (cationic) state of aqueous imidazole. Via PE spectroscopy we characterize its lowest ionization potential, which is distinct from that of the deprotonated form. This finding is supported by *ab initio* NEPCM calculations. Since this is the first application of the NEPCM approach for ionization of aqueous

cations, benchmark calculations are also performed for lithium, sodium, and potassium as simple atomic ions.

2. Methods

Experimental

Photoelectron (PE) spectra of an aqueous solution of imidazole were measured at the U41 PGM undulator beamline of the synchrotron radiation facility BESSY (Berlin), using 200 eV photon energy. The spectra were collected from a 15 μm liquid microjet traveling at a velocity of 120 m/s with a temperature of 4 $^{\circ}\text{C}$. Additional measurements of sulfuric acid in water were performed at the U125 undulator beamline, using 100 eV photon energy. Details of the experimental setup, and a discussion of photoelectron spectroscopy of highly volatile aqueous solutions have been reported previously.^{5,11} 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 micro-jet (operating at 10^{-5} mbar) from the hemispherical kinetic energy analyzer (operating at 10^{-9} mbar). Energy resolution of the beamlines was better than 200 meV at the incident photon energies 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). Electron kinetic energies (eKE) are calibrated based on the $1b_1$ binding energy (BE) of liquid water.¹²

Highly demineralized water was used for preparing the 2m imidazole aqueous solutions. Imidazole (Merck) was used without further purification. The room-temperature pH value of 2.6

was adjusted by titrating the as-prepared imidazole solution (at pH 10.6)¹⁰ with 20 % sulfuric acid. At this pH value 99% of all imidazole molecules are protonated.

Computational

The geometry of the protonated imidazole was optimized at the MP2/aug-cc-pVDZ level, while the corresponding dication was either assumed at the cationic geometry (for VIP evaluation) or optimized (for adiabatic ionization) at the UMP2 level. Fortunately, spin contamination in the case of protonated imidazole dication is not a serious issue (S^2 being below 0.82). Nevertheless, higher spin components have been removed using Schlegel's projected MP2 approach (PMP2).¹³ For each of the protonated imidazole(H_2O)_n ($n = 1 - 5$) clusters geometry optimization was performed at the MP2/aug-cc-pVDZ level of theory. MP2 calculations were also applied to study ionization of aqueous alkali cations. Since we describe core (or sub-valence) ionization in these cases, cc-pCVDZ bases for Li and Na and Feller's CVDZ basis for K have been employed and all electrons have been correlated.

The effect of bulk water on the ionization potential was modeled using the polarizable continuum model (PCM).¹⁴⁻¹⁶ To describe adiabatic ionization, the reaction field was optimized both for the cationic and dicationic species using a standard PCM procedure. To calculate the VIP, we first optimized the reaction field for the protonated imidazole (or protonated imidazole with 1-5 explicit water molecules). In a subsequent step we employed the non-equilibrium solvation protocol as implemented in Gaussian03.¹⁷⁻¹⁹ In this way, the fast electronic component of polarization was accounted for, while the nuclear orientational polarization was not; this approach corresponds to vertical ionization. Since there has not been any benchmarking of non-equilibrium PCM for aqueous cations we performed also calculation of ionization of the series of

simple alkali ions (Li^+ , Na^+ , and K^+), for which experimental values are accurately known both in the gas-phase and in water. By their nature, polarizable continuum models cannot accurately describe the molecular details of solvation within the first shell of water molecules. To quantify the effect of this deficiency on the ionization potential we also used a hybrid model, in which small protonated imidazole-water clusters, described above, were immersed in the polarizable continuum. All *ab initio* calculations were performed using the Gaussian03 program package.¹⁹

3. Results and Discussion

Figure 1 displays the valence photoelectron (PE) spectrum from 2m aqueous imidazole solution of pH 2.6, at which value practically all (>99%) imidazole molecules are protonated. The second PE spectrum in the figure is that of neat liquid water, measured at the same photon energy of 200 eV, which is shown for comparison. Intensities of the two spectra are normalized at the background signal near 35 eV electron binding energy (BE). Note that the overall decrease of the water signal (the four valence orbitals being labeled in Figure 1) for the solution spectrum is due to the lower water concentration in the former system, in agreement with a previous PE study of acidic and basic aqueous solutions.⁷ Solute contributions to the spectrum can be identified by the energies at which the PE intensities of the solution are larger than for pure water. Most pronounced spectral differences are observed in the low-energy region, <11 eV, containing both signals from the highest occupied molecular orbital (HOMO) of protonated imidazole and of HSO_4^- . The latter was used to adjust the pH value of the solution. In order to distinguish the different solute contributions we have also measured the PE of a 20% H_2SO_4 aqueous solution, from which the HOMO of HSO_4^- was determined. The corresponding PE spectrum, measured at 100 eV photon energy, and shown in Figure 2a near the ionization

threshold, reveals a peak maximum of the HSO_4^- HOMO at -9.45 eV, with a 1.3 eV peak width (fwhm). Here we have used one Gaussian to fit the $1b_1$ water peak, which was obtained from the pure water PE spectrum, and another Gaussian to fit the acid signal. With the same two Gaussians (except for intensities) we find the parameters for a third Gaussian accounting for the signal of the HOMO of protonated imidazole, as shown in Figure 2b. Its maximum is at 8.96 eV BE (1.0 eV fwhm), corresponding to the lowest vertical ionization potential (VIP). This energy is by 0.7 eV higher than that for deprotonated imidazole.¹¹

Since the *ab initio* NEPCM approach has not been applied to ionization of aqueous cations yet, we first performed benchmark calculations for simple alkali cations. Calculations of gas-phase ionization potentials show that the MP2 method provides converged results within 0.25 eV from the experimental values (see Table 1). Applying the polarizable continuum solvation model at this level of theory correctly reproduces the large decrease in ionization potential (Table 2), which can be rationalized in terms of stronger solvent stabilization of the nascent dication compared to the parent cation. NEPCM performs quantitatively for the VIP with deviations from experiment decreasing from 0.8 eV for Li^+ to less than 0.3 eV for Na^+ and K^+ . Clearly, the larger the cation and the less tight and structured the solvent shell, the better the performance of the polarizable continuum model. Note that equilibrium PCM, which actually provides adiabatic ionization potentials, underestimates the experimental values of VIP by about 2 eV due to the additional nuclear polarization of the solvent about the dication. Also note that the adiabatic ionization potentials presented in Table 2 differ somewhat from values presented previously,⁶ which is primarily due to reparametrization of the sizes of dielectric cavities done in the newer version of the Gaussian program.¹⁹

After successfully benchmarking the *ab initio* NEPCM approach for sub-valence ionization of aqueous alkali cations, we applied this method to protonated imidazole. In addition to hydrating, in the continuum solvent, the bare cation we also carried out calculations, where we first performed a microhydration of the protonated imidazole with one to five explicit water molecules. We discuss these microhydrated clusters first. The MP2/aug-cc-pvdz optimized structures of protonated imidazole with 0 – 5 water molecules are shown in Figure 3. We see that the first and second water molecules hydrate separately the two acidic hydrogens of the cation. Each of the next two waters makes a hydrogen bond to one of these two water molecules. Finally, the fifth water molecule merges the two hydrating centers, forming a hydrogen-bonding bridge between them. This bridge is somewhat similar to that formed upon microhydrating deprotonated imidazole,¹¹ however, both its endpoints are now formed by hydrogen bonds with oxygen-donating water molecules. Note also the very good agreement with previously determined *ab initio* structures of small clusters with one to two water molecules.^{21,22}

As a next step, we performed *ab initio* NEPCM calculations taking as a solute either the bare protonated imidazole or the above microhydrated clusters. The resulting VIPs are summarized in Table 3. We see that already without explicit water molecules the VIP of aqueous protonated imidazole of 9.21 eV falls within 0.25 eV of the value measured by PE spectroscopy. Upon adding explicit water molecules the VIP obtained using NEPCM decreases slightly, reaching for the largest investigated system almost exactly the experimental value (Table 3). Note that the adiabatic ionization potential for protonated imidazole in an equilibrium polarizable continuum amounts to 7.48 eV. The decrease of 1.73 eV compared to the VIP is due to nuclear polarization of the solvent around the nascent dication.

Note that the gas-phase VIP of protonated imidazole amounts at the MP2/aug-cc-pvdz level to 15.16 eV (Table 3). This is about 6 eV above the corresponding value in water, indicating a large solvent effect (compared, e.g., to the 1 eV solvent-induced shift for deprotonated imidazole¹¹). The large decrease in VIP is due to a much stronger electronic polarization of the solvent around the dication vs the cation, and the *ab initio* NEPCM correctly reproduces this effect. As in our previous study of deprotonated imidazole,¹¹ it turns out that this electronic polarization is of a long range character, therefore, small cluster models cannot quantitatively account for it. It is true that explicit water molecules do lower the VIP, however, the first five water molecules only account for about 2 out of the 6 eV of the solvent induced decrease of the ionization potential of protonated imidazole (see Table 3).

4. Conclusions

The vertical ionization potential of aqueous protonated imidazole, obtained by photoelectron spectroscopy, amounts to 8.96 eV. This value is in an excellent agreement with predictions from *ab initio* calculations employing a non-equilibrium polarizable continuum model, which were also shown to perform quantitatively in benchmarking calculations of ionization of aqueous alkali cations. Upon deprotonation the VIP of aqueous imidazole decreases by 0.7 eV.¹¹ This value is well above the resolution of the experiment and accuracy of the calculation. Since imidazole forms the titratable side chain group of histidine, the present techniques shall be suitable for analyzing the protonation state of this amino acid in various aqueous environments, including hydrated peptides and proteins.

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Table 1: Comparison of MP2/cc-pvdz (MP2/cc-pvtz) and experimental ionization potential (in eV) of the three lightest alkali cations in the gas phase.

Cation	MP2/cc-pvdz (cc-pvtz)	Experimental ionization potential in the gas phase ²⁰
Li ⁺	75.39 (75.46)	75.64
Na ⁺	47.30 (47.24)	47.28
K ⁺	31.27 (31.50)	31.62

Table 2: Comparison of equilibrium and non-equilibrium PCM MP2/cc-pvdz (MP2/cc-pvtz) results with experimental vertical ionization potential (in eV) of alkali cations in water.

Cation	Equilibrium PCM MP2/cc-pvdz (cc-pvtz)	Non-equilibrium PCM MP2/cc-pvdz (cc-pvtz)	Experimental vertical ionization potential in water ⁶
Li ⁺	58.01 (58.08)	61.24 (61.30)	60.4
Na ⁺	33.01 (32.95)	35.67 (35.60)	35.4
K ⁺	20.07 (20.30)	22.16 (22.38)	22.2

Table 3: MP2/aug-cc-pvdz vertical ionization potentials (in eV) of protonated imidazole with 0 – 5 explicit water molecules with or without the aqueous solvent treated within the non-equilibrium PCM approach.

No. of explicit water molecules	VIP with the NEPCM	VIP without the NEPCM
0	9.21	15.16
1	9.11	14.49
2	9.20	13.87
3	9.15	13.55
4	8.93	13.24
5	8.97	13.11

Figure captions

Figure 1: Valence photoelectron spectra of 2m imidazole aqueous solution (at pH 2.6) and of pure liquid water measured at 200 eV photon energy. Peaks arising from ionizing the water valence orbitals $1b_1$, $3a_1$, $1b_2$, and $2a_1$ are labeled. The small narrow peak overlapping with the $3a_1$ emission results from gas-phase water molecules.

Figure 2: Near-ionization-threshold photoelectron spectra and peak fits of a 20% H_2SO_4 aqueous solution (a), and of a 2m imidazole aqueous solution at pH 2.6 (b). Photon energy in (a) and (b) was 100 eV and 200 eV, respectively. Thick black curves are the measured PE spectra, the thin black curves are the Gaussian fit of the water $1b_1$ emission, the green peak is the Gaussian fit to the HSO_4^- HOMO, and the red peak is the Gaussian fit of the HOMO of protonated imidazole. The dotted line is the total fit. The HSO_4^- HOMO binding energy is 9.45 eV, and peak width is 1.3 eV. Protonated imidazole in water has a binding energy of 8.96 eV, and 1.0 eV peak width.

Figure 3: Optimized (at the MP2/aug-cc-pvdz level) structure of protonated imidazole with zero to five water molecules. Only the energetically lowest geometries found are depicted.

Figure 1:

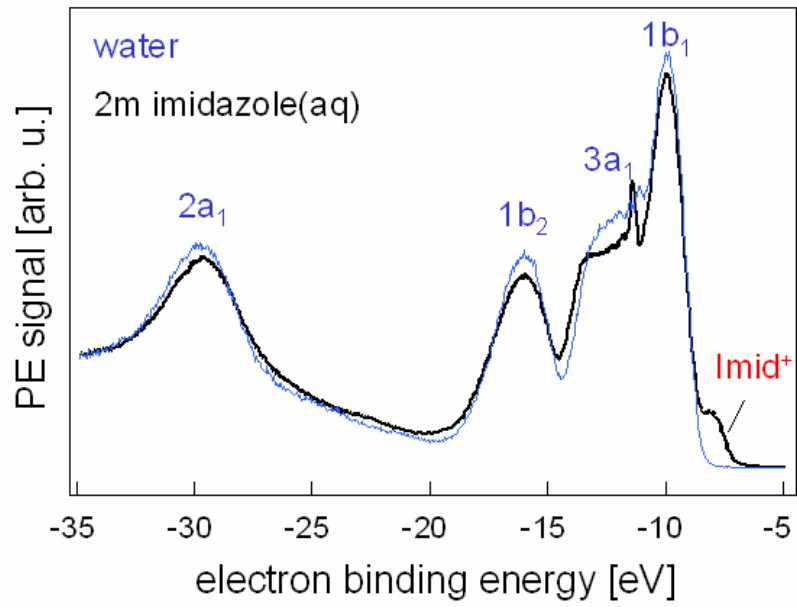


Figure 2:

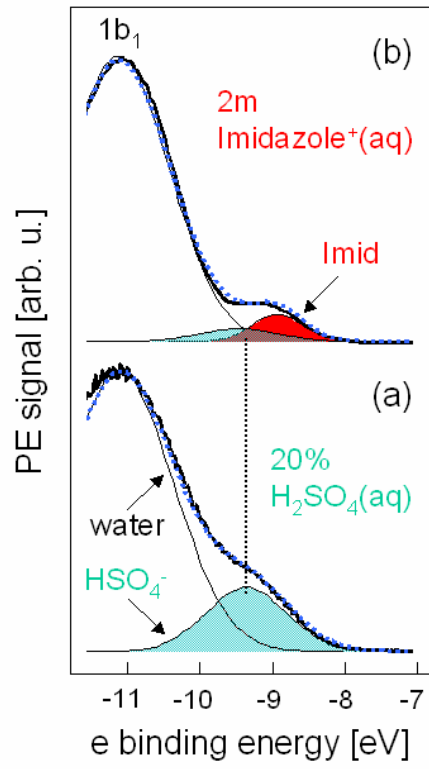


Figure 3:

