The electronic spectrum of protonated adenine: Theory and experiment

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In this work we present the results of a combined experimental and theoretical study concerned with the question how a proton changes the electronic spectrum and dynamics of adenine. In the experimental part, isolated adenine ions have been formed by electro-spray ionisation, stored, mass-selected and cooled in a Paul trap and dissociated by resonant photoexcitation with ns UV laser pulses. The S₀–S₁ spectrum of protonated adenine recorded by fragment ion detection lies in a similar energy range as the first πn* transition of neutral 9H-adenine. It shows a flat onset with a broad substructure, indicating a large S₀–S₁ geometry shift and an ultra-short lifetime. In the theoretical part, relative energies of the ground and the excited states of the most important tautomers have been calculated by means of a combined density functional theory and multi-reference configuration interaction approach. Protonation at the nitrogen in position 1 of the neutral 9H-adenine tautomer yields the most stable protonated adenine species, 1H-9H-A. The 3H-7H-A* and the 3H-9H-A* tautomers, formed by protonation of 7H- and 9H-adenine in 3-position, are higher in energy by 162 cm⁻¹ and 688 cm⁻¹, respectively. Other tautomers lie at considerably higher energies. Calculated vertical absorption spectra are reported for all investigated tautomers whereas geometry optimisations of excited states have been carried out only for the most interesting ones. The S₁ state energies and geometries are found to depend on the protonation site. The theoretical data match best with the experimental onset of the spectrum for the 1H-9H-A* tautomer although we cannot definitely exclude contributions to the experimental spectrum from the 3H-7H-A* tautomer at higher energies. The vertical S₀ → S₁ excitation energy is similar to the one in neutral 9H-adenine. As for the neutral adenine, we find a conical intersection of the S₁ of protonated adenine with the ground state in an out-of-plane coordinate but at lower energies and accessible without barrier.

I. Introduction

The high photostability of DNA is essential for life on earth. The excited state lifetimes of nucleic acid bases are short in solution and in gas phase. Several mechanisms have been discussed to explain this ultrafast deactivation. Most of them involve a coupling between the optically bright and therefore preferentially photo-excited ππ* state and the πn* state. Another model proposes a crossing of the lowest πn* state to a dissociative ππ* state which might recombine to the ground state by the cage effect in solution. In DNA base pairs an alternative route could be a hydrogen or proton transfer between the bases. Very recently, a direct relaxation pathway from the photo-excited ππ* state of 9H-adenine (9H-A) to the electronic ground state has been identified in quantum chemical calculations. It involves a conical intersection at a nuclear geometry where the six-membered ring is strongly puckered. On the πn* potential energy hypersurface (PEH) of 9H-A this structure is separated from the minimum region by a shallow barrier only and can therefore be accessed by vibronic levels close to the origin.

Probing the first steps of the deactivation pathway in solution directly is a great challenge. From the bi-exponential decay of photo-excited adenine in aqueous solution (corresponding to lifetimes of 100 fs and 1 ps, respectively) it was concluded that either more than one electronic state or more than one tautomer is involved. Fluorescence spectra of neutral adenine were measured by Longworth et al. Later they were identified by Wilson and Callis to originate from the 7H-adenine (7H-A) tautomer which is a minor component in solution at pH 7 (6%, 22%, 22%). The explanation for the approximately 200 times lower fluorescence yield of 9H-A is a fast non-radiative decay of its first excited πn* state. Optical spectroscopy in isolated systems is able to provide vibrationally resolved spectra. In combination with quantum chemical calculations these spectra may provide information about the energetic position and the geometric as well as the electronic structures of excited electronic states. Possibly the spectral pattern might allow the identification of the tautomer species. High-resolution S₀–S₁ spectra of neutral adenine were studied in molecular beam experiments by de Vries, Kleinermanns and coworkers as well as Kim and coworkers. They assigned vibronic transitions to the 9H-A and 7H-A tautomers and were able to distinguish singlet nπ* and πn* transitions. Lifetimes of isolated adenine have been determined with femtosecond pump–probe experiments and band contour analyses. Vibronic peaks close to the origin of the πn* transition exhibit lifetimes in the picosecond range while lifetimes of the order of 50 fs have been reported at higher energies. Optical spectroscopy combined with mass detection allows the direct measurement of photoproducts. For example in neutral adenine, hydrogen atom loss was detected at high S₁ energies.

While under physiological conditions the nucleic acid bases typically exist in their neutral forms, there is evidence that protonated adenine is involved in the acid–base mediated phosphodiester cleavage in the hairpin ribozyme of catalytic RNA. Addressing the question of photostability,
protonated adenine coupled to a deoxyribose-5'-monophosphate (AMP) was shown to exhibit a fragmentation behaviour after irradiation with 266 nm photons that differs substantially from the one of neutral adenine coupled to a deoxyribose-5'-monophosphate anion. While the latter complex decomposes statistically, a non-ergodic cleavage of the glycosidic N9-C bond is observed for a large portion of the protonated AMP cations. Obviously protonation substantially changes reactivity after photoexcitation. It appears therefore necessary to understand how the electronic structure of photoexcited adenine is perturbed by the proton from that of neutral adenine.

The spectroscopy of protonated species in solution is principally complicated because of solvation shifts and inhomogeneous broadening. In addition charged and neutral species coexist leading to a superposition of spectra. In polar protic solvents proton exchange is fast, causing lifetime broadening. For most of the nucleic acid bases the spectra of different tautomers superimpose and make the spectra congested.

One important advantage of a gas phase experiment is that mass selection prior to spectroscopy guarantees that one does not deal with dimers, aggregates or chemically modified samples and that all adenine molecules investigated are protonated. Freiser and Beauchamp were the first to perform electronic excitation spectroscopy on protonated species in the gas phase. They generated the protonated aromats by chemical ionisation. Other experiments on protonated aromats used a discharge for the formation of the protonated species and applied IR spectroscopy. Molecules of biological relevance are more contractedly in excited states of protonated species and the electronic wave functions are more contracted than in the neutral molecule. For the three most stable protonated adenine tautomers (see above) can be protonated at different sites. In addition charged and neutral species coexist leading to a superposition of spectra. In polar protic solvents proton exchange is fast, causing lifetime broadening. For most of the nucleic acid bases the spectra of different tautomers superimpose and make the spectra congested.

Protonated adenine has many tautomeric forms. This complexity arises from the fact that the two stable neutral adenine tautomers (see above) can be protonated at different sites. In 1951 crystallographic evidence was found that 9H-A is protonated preferentially at the nitrogen atom in position 1 of the purine ring. Experimental estimates of the proton affinity of 9H-A range from 938 kJ mol⁻¹ to 943 kJ mol⁻¹ in the gas phase. To our knowledge, protonation of 7H-A has not been investigated so far.

Ground state properties of neutral and protonated species can be computed with high accuracy. For example, Chen et al. calculated the proton affinity of the most stable neutral adenine tautomer 9H-A in good agreement with experimental data. For molecules of this size, the quantum chemical treatment of electronically excited states with comparable accuracy is a challenge, even more so if excited state geometry optimisation has to be performed. Such calculations are, however, important for gaining a detailed insight into the physicochemical energy relaxation mechanisms. To our knowledge, the number of theoretical calculations performed on excited states of protonated species is sparse although protonated organic molecules typically are electron-electron systems and the electronic wave functions are more contracted than in the neutral molecule. For the three most stable protonated tautomers derived from 9H-A, Chen et al. calculated vertical spectra by means of time-dependent density functional theory. However, the main focus of the latter work lies on bond dissociation energies rather than spectroscopy. Especially the character of the electronically excited states was not given.

In this paper we raise the question how protonation at different sites influences the electronic spectrum and dynamics of adenine in the gas phase. To this end, we have recorded spectra of protonated adenine in solution and in a molecular beam. The interpretation of the measured solution and gas phase data is aided by quantum chemical calculations on the ground and excited states of various tautomers of protonated adenine employing a combined density functional theory/multi-reference configuration interaction (DFT/MRCI) method.

II. Experimental results

II.1 The spectrum of protonated adenine in solution

In Fig. 1 the UV–Vis spectrum of dilute (5 × 10⁻⁵ molar) solutions of adenine in ethylene glycol–water (1:1) is shown at pH 2 (solid line) and pH 7 (dashed line) for comparison with results on isolated adenine (see section II.2). The spectra were recorded with a conventional differential two-beam UV–VIS spectrometer at room temperature. At pH 7 most of the adenine molecules are neutral (ratio of neutral to protonated: 1000:1) and at pH 2 most of the adenine molecules are protonated (ratio of neutral to protonated: 1:100). At pH 2 and pH 7 the low-energetic absorption bands of adenine have similar integral intensities and are unstructured. The lack of vibronic structure could be caused by solvation and/or intra-molecular effects. Gas phase experiments of neutral adenine show sharp vibronic transitions in the low-energy and increasingly broad spectra in the high-energy range. In glassy ethylene glycol–water mixtures at 77 K also a distinct origin band at approximately 280 nm is observed. We therefore suppose that the absence of vibrational structure in our solution spectrum (Fig. 1) is caused by a temperature and solvent broadening for the neutral adenine. For the protonated adenine the first band in the UV spectrum shows a small red-shift and a less steep onset compared to the spectrum of the neutral form (Fig. 1). This red-shift of 2 nm upon protonation has been reported by Mason in the 1950s. In solution or matrix one cannot tell whether the red-shift is due to changes of intramolecular properties upon protonation or a different solvation of the protonated species in S₀ and S₁. The low-energetic band in Fig. 1 could in principle be due to several electronic states, as suspected for neutral adenine. Its shape would then be influenced by the transition moments and the relative positions of the electronic states. However, one even does not know whether the spectrum of the protonated species originates from the same tautomer or a superposition of the absorptions of two tautomers. To address these questions, we recorded gas phase S₀–S₁ spectra of protonated adenine and performed quantum chemical calculations.

![Absorption spectrum of adenine (5 × 10⁻⁵ molar) in ethylene glycol–water (1:1) at pH 7 (dashed line) and pH 2 (solid line). At pH 2 most of the adenine molecules should be protonated. Note the red-shift and the smooth rising slope upon protonation. From this solution spectrum it is unclear whether the spectral differences are due to intramolecular effects caused by protonation or a change in S₀ versus S₁ solvation caused by protonation.](image-url)
II.2 Experiments on isolated protonated adenine

II.2.1 Experimental set-up. The experimental set-up is described elsewhere. In short, the experiments have been carried out using a modified commercial ESI mass spectrometer (Esquire 3000, Bruker Daltonik) and a pulsed excimer-pumped ns frequency-doubled dye laser. The adenine sample was purchased from Sigma and used without further purification. It was dissolved in a mixture of water, acetonitrile and acetic acid (33%, 66%, 1%, pH = 2.7) and sprayed in a sheathflow-supported electrospray process. The ions are transported through a capillary and several pumping stages into the ion trap. For efficient ion transfer through the first two pumping stages the ions are accelerated. Because they still undergo collisions, the ion internal energy at this point is for some molecules even high enough for fragmentation. This heating could influence the tautomer distribution as formed by the ES process, but this is improbable because two protons have to be moved for tautomerisation in gas phase. In the third vacuum chamber the ions are guided in an octopole into the fourth chamber and the ion trap. Ion storage, mass selection, fragmentation and mass analysis were performed in a Paul trap, a quadrupole radio frequency ion trap. The trap was filled with He (pressure in the trap: ~0.1 mbar) and the electrodes cooled by liquid N2. Selection of one mass is performed by applying time-dependent radio frequency to the end-caps of the Paul trap. After this, typically 100 ions of the mass of interest remain in the trap. They were then allowed to cool down for some ms before laser interaction (one laser shot only). Internal cooling of the ions is expected to take place by ion-He collisions. The final ion internal temperature is not known and difficult to determine.

To avoid pick-up of solvent molecules, mass analysis of the photo-fragments and parent ions follows directly the laser-ion interaction. Mass analysis was performed by application of a RF voltage to the end-caps and an increase of the trapping RF voltage. For detection the ejected ions are converted to electron multipliers, which are then amplified by a channeltron. The single ion signals are amplified and counted by gated counters. The S0–S1 spectrum of adenine is recorded by scanning the laser wavelength and simultaneous recording the fragment ion intensity. The total ion intensity was also monitored for supervision. 20 measurements were summed for each wavelength data point. The total number of ions and the laser intensity were held constant within a limit of ±10% during the whole spectrum.

II.2.2 The S0–S1 photofragment spectrum of protonated adenine: The lowest ππ* transition. The photoabsorption and subsequent dissociation of adenine in the UV is efficient and leads to loss of NH3. No other fragment is observed, especially no loss of a hydrogen atom or a proton. The NH3 dissociation channel is open after absorption of one UV photon. The UV laser excitation spectrum of adenine as shown in Fig. 2a was recorded by monitoring the total fragment ion intensity in dependence on the laser wavelength. The previously published spectrum of protonated tryptophan (Fig. 2b) is displayed for comparison. Both molecules absorb in a similar wavelength range and with a similar cross section. During recording the spectra laser intensity, temperature inside the trap, and ion number was kept constant. Under these conditions the spectra are fully reproducible.

The onset of the spectrum of protonated adenine (Fig. 2a) is red-shifted with respect to the origin transition of the ππ* state of neutral adenine. The spectral structure shows a smooth onset (note that due to this it is difficult to accurately determine the band onset) and some broad structures at higher energies.

In contrast, the photodissociation spectrum of tryptophan (Fig. 2b) exhibits a distinct origin band and a gap, which is interpreted as the gap between the S1 origin transition and the in-plane indole chromophore vibrations. For both molecules the cooling in the Paul trap was performed under the same conditions. We therefore rule out different broadening due to different internal temperatures.

The indole and purine chromophores are both planar double ring structures of similar size. The fact that we observe a distinct origin band in the tryptophan spectrum shows that in principle we would be able to resolve the origin of the S1 state of adenine if it had sufficient transition moment and was spectrally isolated. Assuming that the S0–S1 Franck–Condon factors would be similar in both molecules, one also expects a gap between the origin transition and the symmetric ring vibrations to appear in adenine, which is not the case. Note that for tryptophan the broadening of the first peak, which contains the origin transition, is attributed to a superposition of hot-band transitions and transitions involving the low-energetic ring-tail vibrations. Because adenine is a stiff molecule without flexible side groups its ππ* origin transition should be even sharper and the gap between the origin and the ring vibrations should be more pronounced than in tryptophan. Therefore there is a distinct difference between the absorptions in protonated tryptophan and protonated adenine. The possibilities to explain the smooth onset and the broad vibronic structure in the spectrum of protonated adenine are: (a) a superposition of several tautomers, (b) a large S0–S1 geometry shift, or (c) an ultra-short lifetime. These possibilities will be discussed on the basis of theoretical results in section IV. The experimental results alone do not rule out one of the three above possibilities.

III. Quantum chemical calculations

III.1 Theoretical methods and technical details

Minimum geometries and harmonic vibrational frequencies of the electronic ground states of protonated adenine tautomers...
were determined at the restricted Kohn–Sham level (B3-LYP functional\cite{55-57}) utilising the Turbomole package.\cite{58} Geometry optimisations of electronically excited singlet states were carried out at the level of time-dependent density functional theory (TDDFT).\cite{59} For all elements, TZVP (valence triple zeta plus (d,p) polarization) basis sets from the Turbomole library were applied.\cite{60} At the optimised ground and excited state geometries, single-point DFT/MRCI calculations were carried out. The principal concept of this approach formulated by Grimme and Waletzke\cite{61} is to describe major parts of dynamic electron correlation by density functional theory whereas static correlation effects are accounted for by MRCI expansions. The configuration state functions (CSFs) in the MRCI expansion are built up from Kohn–Sham (KS) orbitals. Diagonal elements of the effective DFT/MRCI Hamiltonian are constructed from the corresponding Hartree–Fock-based expression and a DFT specific correction term. In the effective DFT/MRCI Hamiltonian, in total five empirical parameters are employed. These parameters depend only on the multiplicity of the calculated state, the number of open orbitals of an electron configuration, and the applied density functional, but do not depend on the atoms or the type of molecule. Currently, optimised parameter sets are available in combination with the BH-LYP functional.\cite{55,62} A common set of reference CSFs is used for all spatial symmetries. The initial set can be generated automatically in a complete active space (CAS)-like procedure and is then iteratively improved. The MRCI expansion is kept short by extensive configuration selection. For further details, we refer to the original publication by Grimme and Waletzke.\cite{61}

In the MRCI step, twelve roots of singlet and triplet multiplicity were determined, respectively. The reference space comprised approximately 80 CSFs for all states of one multiplicity. Only the inner shells (1s electrons at carbon and nitrogen) were treated as frozen core. The dimension of the actual MRCI space including energy-selected single and double excitations out of the reference space ranged from about 20 000 to 100 000 CSFs, depending on the specific conformer and the molecular symmetry.

For the DFT/MRCI the root mean square deviation for all states including the Rydberg states is typically less than ±0.15 eV.\cite{61} Note that for TDDFT the typical error is 0.58 eV.\cite{54}

III.2 Tautomer energies

Starting from the two most stable tautomers of neutral adenine, 9H-A and 7H-A, various tautomers of protonated adenine can be constructed. For each of the investigated protonated adenine tautomers, we find a Cs symmetric equilibrium nuclear arrangement where all carbon and nitrogen atoms lie in one plane. The positive second derivatives of the potential energy with respect to the normal coordinates confirm that these structures correspond to true minima of the ground state PEH. The relative energies of the investigated tautomers including zero-point vibrational energy (ZPVE) corrections are presented in Fig. 3. As purine constitutes a conjugated π system, the breaking of a double bond disturbs the conjugation and is unfavourable. To a smaller extent, the same is true for a protonation at the amino group. This leaves the lone-pair (n) electrons in the rings as the best available ones for the formation of a dative σ bond with the proton.

The most stable tautomer is obtained when the proton is attached to 9H-A in N1-position. Our calculated protonation energy (943 kJ mol⁻¹ including ZPVE corrections) is in excellent accord with experimental values (938–943 kJ mol⁻¹)\cite{49,50} and a recent theoretical result (939 kJ mol⁻¹) by Chen et al.\cite{51} The charge, which is introduced by the surplus proton, is delocalised over all N–H groups in the product. As a result, the tautomers 1H-9H-A and 1H-9H-A⁺ cannot be distinguished and we therefore term them 1H-9H-A⁺. Similar considerations hold for the other tautomers. Interestingly, protonation of 7H-A in N1-position (3H-7H-A⁺) yields an almost equally stable tautomer (162 cm⁻¹ above 1H-9H-A⁺). This tautomer has not been investigated in ref. 51. Note that the 7H-A tautomer of neutral adenine is located 2678 cm⁻¹ (32 kJ mol⁻¹) higher than the 9H-A tautomer at the same level of theory, but exhibits a larger protonation energy (978 kJ mol⁻¹) than the latter. Protonation of 9H-A in N1-position (3H-9H-A⁺) forms the third tautomer in energetic order (688 cm⁻¹ above 1H-9H-A⁺, in line with the results of Chen et al.\cite{23}) All other tautomers are situated at markedly higher energies.

For neutral adenine the 7H-A has a considerably larger dipole moment than the 9H-A and therefore is more stabilized in polar solvents than the 9H-A. To test whether there is a similar solvent effect for protonated adenine, we investigated for the three lowest-energetic tautomers the stabilities of various clusters in which one water molecule is hydrogen-bonded to protonated adenine. The equilibrium of the tautomers in water may play a role for the tautomer distribution formed in the ESI desolvation process (see section IV). Not astoundingly, we find only clusters in which the protonated...
adenine acts as the hydrogen bond (HB) donor and the water oxygen as the HB acceptor. The strengths of single HBs range from about 3800 cm\(^{-1}\) (45 kJ mol\(^{-1}\)) to 4500 cm\(^{-1}\) (54 kJ mol\(^{-1}\)), including ZPVE corrections. In these complexes, the water molecule lies in the molecular plane of the purine ring. However, for the 1H-9H-A\(^+\) and 3H-7H-A\(^-\) tautomers, we find one outstanding complex each in which water forms a bridged HB and is oriented perpendicular to the adenine molecular plane. In 1H-9H-A\(^+\), the oxygen attaches to the H-N\(_2\) and the neighbouring (exo) amino H-N bond. The HB strength of this cluster is 5608 cm\(^{-1}\) (67.1 kJ mol\(^{-1}\)) including ZPVE correction. 3H-7H-A\(^-\) can form a bridged HB employing the H-N\(_2\) and the neighbouring (endo) amino H-N bond. The HB strength is slightly smaller in this case (5496 cm\(^{-1}\) = 65.7 kJ mol\(^{-1}\)). Thus, complexation with one water molecule will shift the equilibrium further towards the 1H-9H-A\(^+\) tautomer. The 3H-9H-A\(^+\) tautomer is not stabilized to the same extent (4751 cm\(^{-1}\) = 56.8 kJ mol\(^{-1}\)) because the N\(_3\)-H and N\(_3\)-H bonds are too far apart to act simultaneously as HB donors. Although bridged hydrogen bonds exist in 1H-7H-A\(^-\)-water clusters, the energy gain does not compensate for its considerably lower tautomer stability.

### III.3 Vertical absorption spectra and characterisation of excited states

In order to investigate the sensitivity of the S\(_0\)-S\(_1\) transition on the protonation and the protonation site we calculated the vertical transition energies of all protonated tautomers and compare them to the calculated energies of the two neutral 9H-A and 7H-A species.

In the following we first review the neutral adenine tautomers. The energetic order of the low-lying excited electronic states of both tautomers is highly sensitive to small geometry changes. The 9H-A tautomer exhibits a planar equilibrium geometry of both tautomers is highly sensitive to small geometry changes. The 9H-A tautomer exhibits a planar equilibrium state for its considerably lower tautomer stability.

Table 1 Vertical and adiabatic excitation energies \(\Delta E_{\text{vert}}\) of the lowest singlet \(\pi^*\) and \(\pi^*\) states of selected tautomers of neutral and protonated adenine as well as absorption dipole oscillator strengths \(f(r)\), calculated at the DFT/MRCI level of theory

<table>
<thead>
<tr>
<th>Species</th>
<th>First singlet (\pi^*) state</th>
<th>First singlet (\pi^*) state</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\Delta E_{\text{vert}})</td>
<td>(f(r)_{\text{vert}})</td>
</tr>
<tr>
<td>9H-Adenine</td>
<td>4.91</td>
<td>0.034</td>
</tr>
<tr>
<td>7H-Adenine</td>
<td>4.81</td>
<td>0.121</td>
</tr>
<tr>
<td>1H-9H-A(^+)</td>
<td>4.83</td>
<td>0.173</td>
</tr>
<tr>
<td>1H-9H-A(^+)</td>
<td>4.83</td>
<td>0.138</td>
</tr>
<tr>
<td>3H-7H-A(^-)</td>
<td>4.90</td>
<td>0.384</td>
</tr>
<tr>
<td>3H-9H-A(^+)</td>
<td>4.76</td>
<td>0.383</td>
</tr>
<tr>
<td>7H-9H-A(^+)</td>
<td>4.39</td>
<td>0.215</td>
</tr>
<tr>
<td>1H-7H-A(^-)</td>
<td>4.80</td>
<td>0.221</td>
</tr>
<tr>
<td>2H-9H-A(^+)</td>
<td>2.31</td>
<td>0.128</td>
</tr>
</tbody>
</table>

\(a\) Electronic structure is an almost equal mixture of \(\pi^*\) and \(\pi^*\) character. \(b\) Energetic location of conical intersection with electronic ground state is displayed. \(c\) Not calculated. \(d\) Energy at planar S\(_2\) (\(\pi^*\)) saddle point; unconstrained geometry optimisation of S\(_2\) did not converge. \(e\) Energy at T\(_1\) (\(\pi^*\)) minimum; geometry optimisation of S\(_2\) converges toward (n – 1) \(\rightarrow\) \(\pi^*\) excitation. \(f\) Minimum could not be located. Energy at conical intersection between singlet \(\pi^*\) and \(\pi^*\) is displayed.
the most stable tautomers of protonated adenine are discussed first excited n*p-state and (ii) the fact that the shift of the first n*p-transition in 1H-9H-A*.

The second pp-membered ring, the n*p-state is the lowest-energetic singlet state. The n orbital of the latter tautomer is localised to a large extent at the N1 centre whereas the density of the n*p LUMO is mainly concentrated on the C2 and C8 centres and the asymmetric σ-bonding orbital of the CH2 group. The extreme red-shift of the first n*p-transition of this tautomer is (i) caused by the high electronic ground state energy and (ii) the fact that the first excited n*p-state is the lowest of all states in all investigated tautomers (vertical absorption energy: 2.04 eV). Because of (ii) this tautomer could probably be formed after photo-excitation. Its excited state potential energy surface is therefore investigated in detail, too.

In the following, the calculated vertical excitation spectra of the most stable tautomers of protonated adenine are discussed in greater detail. The lowest singlet n*p-state of 1H-9H-A* originates from the HOMO → LUMO transition, in contrast to neutral 9H-adenine where this excitation leads to the second singlet n*p-state. It is predicted to occur at about 4.83 eV in the vertical spectrum of 1H-9H-A*, markedly red-shifted with respect to the corresponding transition in 9H-A at 5.09 eV. The second n*p (HOMO → LUMO + 1) absorption at 5.15 eV experiences a distinct blue-shift with respect to neutral 9H-A.

Both transitions are strong, as expected for n*p-excitations. The sum of their oscillator strengths is of similar size as the combined oscillator strengths of the first two n*p-transitions in neutral 9H-A which agrees well with the ratio of the band intensities in the solution spectra in Fig. 1. The third (weak) transition at 5.23 eV originates from an n*p excitation. Its blue-shift amounts to approximately 0.2 eV. It is difficult to compare these values with the TDDFT results of Chen et al. because these authors did not specify the electronic structure of the excited states. They find the lowest singlet transition at 4.89 eV, two near-degenerate singlet states at 5.06 and 5.08 eV, respectively, and a fourth singlet state at 5.19 eV. The energy range thus compares well with our results. In 1H-7H-A*, the HOMO → LUMO transition at 4.77 eV (first excited 1n*p state) is almost unaffected by the protonation. It is noteworthy that in this tautomer the 1n*p state is located below 1n*p (see Table 1).

Proton attachment of 9H-A to the N1-position causes a strong red shift of the HOMO → LUMO transition from 5.09 eV in 9H-A to 4.76 eV in 3H-9H-A*, with the effect that this electronic structure dominates the S2 state of this tautomer. The oscillator strength of this transition is of the same size as the combined oscillator strengths of the first two n*p-transitions in 1H-9H-A*. The S2 state at 5.13 eV exhibits n*p character followed by a n*p transition with medium oscillator strength at 5.18 eV. Without giving an electronic characterisation Chen et al. reported singlet states at 4.79 eV, 5.06 eV, and 5.27 eV for this tautomer, which agree well with our data.

The 3H-7H-A* tautomer was not investigated by Chen et al. Its first vertical n*p-absorption lies at slightly shorter wavelengths (4.90 eV) than the corresponding vertical transition in 7H-A (4.81 eV). Its oscillator strength is very strong and comparable to the one in 3H-9H-A*. The second transition in the vertical absorption spectrum of this tautomer yields a singlet n*p-state with medium oscillator strength at 5.14 eV. The weak transition at 5.27 eV corresponds to the first singlet n*p. Attaching the proton to the free nitrogen lone-pair in the five-membered ring (7H-9H-A*) leads to an appreciable decrease of the first n*p excitation energy with respect to both, 7H-A and 9H-A. In this tautomer, the lowest n*p absorption band is predicted to peak at about 4.39 eV. The n*p-singlet for which we find an excitation energy of 4.49 eV is nearly unaffected by protonation at this site. Chen et al. find considerably lower excitation energies (4.17 eV, 4.24 eV) in their TDDFT calculations for this tautomer.

III.4 Adiabatic spectra of selected tautomers

The lack of an isolated origin transition in the resonant photofragment spectrum of adenine (section II.2.2) and the observation of broad peaks points toward a large geometry change upon S0–S1 excitation and a short lifetime. To account for relaxation effects, we optimised the nuclear arrangements in S1-states of the most interesting tautomers (1H-9H-A*, 3H-7H-A*, 3H-9H-A*, 7H-9H-A*, 1H-7H-A*, and 2H-9H-A*) by means of TDDFT and carried out single-point DFT/MRCI calculations at the minimum structures. No symmetry constraints were imposed in these steps. The adiabatic excitation energies of the lowest-lying singlet n*p and n*p states are shown in Table 1 together with the corresponding vertical excitation energies at the ground state minimum geometry. Dramatic relaxation effects are found for nearly all protonated adenine tautomers.

When the geometry parameters of the n*p-excited 1H-9H-A* tautomer are optimised under planarity constraints, significant increases in bond length are found in the six-membered ring (Table 2), except for the N3-C6 bond that actually shrinks by almost 0.05 Å. In this way, the destructive overlap of amplitudes in the anti-bonding n MO is diminished. Despite the pronounced bond length changes, the energy release after vertical absorption is small (0.25 eV). DFT/MRCI calculations place the planar n*p structure at 4.58 eV (vertical excitation energy: 4.83 eV). However, as the determination of harmonic frequencies at this stationary point shows, the planar n*p structure represents a saddle-point on the excited PEH. Lifting the symmetry constraints yields a nuclear arrangement with a strongly puckered six-membered ring (Fig. 5 right structure). The out-of-plane distortions of the C2 centre and the associated H atom are accompanied by a further elongation of the C3-C6 bond. Note also the short bond distance between the purine ring and the amino nitrogen that is indicative of a distinct double bond character. The puckering of the six-membered ring leads to a further decrease of the excited state potential energy by about 0.5 eV. During this relaxation of the excited state a step increase of the electronic ground state PEH (Fig. 5, right) is observed. Concomitantly, the S0 and S1
Table 2 Geometry parameters obtained from (TD)DFT calculations on electronic ground and excited states of 1H-9H-A\(^*\) and 3H-7H-A\(^*\) employing the B3LYP functional

<table>
<thead>
<tr>
<th>Bond</th>
<th>(\text{GS}_{\text{min}})</th>
<th>(\text{i}^\pi\pi^*_{\text{pl}})</th>
<th>(\text{i}^\pi\pi^*_{\text{CI}})</th>
<th>(\text{i}^\pi\pi^*_{\text{p}^{\dagger}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1-C2</td>
<td>1.383</td>
<td>1.413</td>
<td>1.413</td>
<td>1.420</td>
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<tr>
<td>C2-N2</td>
<td>1.293</td>
<td>1.365</td>
<td>1.375</td>
<td>1.325</td>
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<tr>
<td>C2-C3</td>
<td>1.347</td>
<td>1.290</td>
<td>1.301</td>
<td>1.282</td>
</tr>
<tr>
<td>C3-C4</td>
<td>1.398</td>
<td>1.474</td>
<td>1.442</td>
<td>1.471</td>
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<tr>
<td>C4-C5</td>
<td>1.401</td>
<td>1.387</td>
<td>1.434</td>
<td>1.379</td>
</tr>
<tr>
<td>C5-C6</td>
<td>1.369</td>
<td>1.382</td>
<td>1.387</td>
<td>1.380</td>
</tr>
<tr>
<td>N5-C6</td>
<td>1.369</td>
<td>1.358</td>
<td>1.361</td>
<td>1.366</td>
</tr>
<tr>
<td>N5-C7</td>
<td>1.308</td>
<td>1.321</td>
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<td>1.290</td>
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<tr>
<td>C7-C8</td>
<td>1.381</td>
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<td>1.384</td>
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<tr>
<td>C8-N9</td>
<td>1.327</td>
<td>1.340</td>
<td>1.303</td>
<td>1.338</td>
</tr>
</tbody>
</table>

\(\text{GS}_{\text{min}}\) (a) Saddle-point, optimised under \(C_s\) symmetry constraints. \(^*\) Not fully converged.

PEHs undergo a conical intersection about 4.1 eV above the planar 1H-9H-A\(^+\) ground state minimum, i.e., about 0.75 eV below the vertical \(1\pi\pi^*\) absorption energy. No barrier has been detected separating the vertical excitation region from the conical intersection. A qualitative explanation for the lack of a barrier and the earlier energetic accessibility of the conical intersection in 1H-9H-A\(^+\) compared to neutral 9H-adenine is easily at hand. Protonation in N1 position weakens the double bond character of the N1–C2 bond (reflected by a bond elongation from 1.34 Å in the electronic ground state of the neutral molecule to 1.38 Å in 1H-9H-A\(^+\)) and thus eases out-of-plane distortions of the six-membered ring. The consequences of the large geometry changes and of the presence of a conical intersection for the experimental band shape are discussed in more detail in section IV.

The minimum of the first excited singlet \(n\pi^*\) state of 1H-9H-A\(^+\) could not be located due to technical reasons. Pre-optimization under symmetry constraints (where \(\pi\pi^*\) and \(n\pi^*\) states are not allowed to mix) yields a nuclear geometry (Table 2) for which the singlet \(n\pi^*\) state is situated energetically about 0.33 eV below the singlet \(n\pi^*\) (Fig. 5, left). However, these states begin to interact when the symmetry constraints are lifted and the TDDFT gradient of the first excited state leads the system eventually toward the above described conical intersection. As the \(n\pi^*\) state is only the third singlet in the vertical absorption spectrum and has a small electric dipole transition probability, the exact location of its minimum is of minor importance and was not calculated.

In DNA, adenine is attached to the phosphate backbone via a deoxycytosine unit in 9-position. To find out whether a conical intersection between singlet \(\pi\pi^*\) and the electronic ground state may also occur in the protonated adenosine, we carried out calculations on a model substance, 9-methyl adenine (9Me-A) that had been protonated in 1-position (1H-9Me-A\(^+\)). From experiments on neutral 9H-A and 9Me-A in solution and in gas phase it is known that substitution of the H-atom in 9-position for a methyl group causes only a minor red-shift of the otherwise similar spectrum. The comparison of the vertical and adiabatic excitation energies of 1H-9Me-A\(^+\) and 1H-9H-A\(^+\) in Table 1 shows the same trend, i.e., the methyl group has only a minor influence on the spectral properties of the protonated adenosine chromophore. In particular, the conical intersection is located at comparable energies.

The PEH of the lowest \(1\pi\pi^*\) state of the second most stable tautomer (3H-7H-A\(^+\)) is very flat in the FC region and the TDDFT gradient is difficult to converge in \(C_1\) symmetry when employing the B3LYP functional. At this level of theoretical treatment, the purine rings remain nearly planar whereas the amino group is slightly pyramidal (Fig. 6). At the relaxed nearly planar nuclear geometry, \(1\pi\pi^*\) constitutes the first excited singlet state with an adiabatic DFT/MRCI excitation energy of 4.72 eV. Despite the moderate energy release during relaxation, we find pronounced bond length changes with respect to the ground state equilibrium geometry (Table 2). In particular, the C2–N2 and C2–C3 bonds in the six-membered ring are markedly elongated whereas the five-membered ring is nearly unaltered. However, as stated already above, the potential well of this state at the planar geometry is not very deep. A small out-of-plane distortion was sufficient to overcome a barrier and direct the geometry gradient toward a conical intersection with the electronic ground state. At the crossing, the six-membered ring is puckered and the C2–H bond is nearly perpendicular to the former molecular plane that is retained in the five-membered ring (see Fig. 6). The geometry thus closely resembles the one of 1H-9H-A\(^+\) at the conical intersection. Energetically, the intersection is situated at about 4.5 eV in 3H-7H-A\(^+\), substantially higher than in 1H-9H-A\(^+\).

At the optimised \(1\pi\pi^*\) geometry of the 3H-7H-A\(^+\) tautomer the amino group is twisted out of plane. Here, the \(n\pi^*\) becomes the lowest singlet state with an adiabatic excitation energy of 4.64 eV (Fig. 6, left) whereas it is situated above the \(1\pi\pi^*\) state in the vertical absorption region and at the

Fig. 5 Relaxation of the first excited singlet \(n\pi^*\) and \(\pi\pi^*\) states of the 1H-9H-A\(^+\) tautomer after photoexcitation. Note the non-planar nuclear arrangement of 1H-9H-A\(^+\) at the conical intersection between the first excited singlet \(n\pi^*\) state and the electronic ground state. The singlet \(n\pi^*\) minimum could not be located due to convergence problems. The excitation energies at the planar saddle point structure are hatched. The electronic spectrum of protonated 9-methyladenine(1H-9Me-A\(^+\)) is very similar. Its singlet \(n\pi^*\) vertical excitation energy amounts to 4.80 eV, the conical intersection is situated at 4.14 eV.
optimised geometry of the flat $\pi\pi^*$ minimum. Consequently, the corresponding PEHs are supposed to cross somewhere. The twisted conformation at the $\pi n^*$ minimum is unfavourable for the $\pi\pi^*$ (HOMO–LUMO) electronic structure, shifting this state to 5.43 eV. It may therefore be assumed that the crossing between $\pi\pi^*$ and $\pi n^*$ takes place well above the corresponding band origins. Because of its small oscillator strength we do not expect to see the $\pi n^*$ transition in the experimental spectrum.

The 3H-9H-A$^+$ ions which constitute the third most stable tautomer appear to retain their planar symmetry in the $\pi\pi^*$ excited state. Bond length relaxation leads to energy decrease on the S$_1$ PEH by about 0.4 eV to 4.35 eV. The convergence of the TDDFT gradient is very slow and eventually finds a minimum for a structure where the nuclei are deflected out-of-plane by less than 1°. As for 3H-7H-A$^+$, the C$_2$–N$_3$ and C$_5$–C$_6$ bonds are considerably stretched. Because its transition dipole is very strong and the planarity is more or less conserved, we would supposedly see the origin of the $\pi\pi^*$ absorption band although the 0–0 transition is not expected to exhibit the largest FC factor. However, the vibronic spectrum of 3H-9H-A$^+$ is predicted to break off shortly above the origin. When we restarted the minimum search from a nuclear arrangement in which the amino group had been pyramidalised, the TDDFT gradient of the S$_1$ state led the molecule toward a conical intersection between singlet $\pi\pi^*$ and the electronic ground states. The energy at the crossing is practically identical to the S$_0$ minimum energy while the nuclear structure at the electronic ground state. At the singlet $\pi n^*$ minimum, the amino group is considerably twisted while the purine rings retain their planar conformation.

IV. Discussion

In the ideal case the comparison between experimental and theoretical results allows the experimentalist to understand the spectra and the theorist to confirm the calculations. Interesting data to compare are here the position and structure of the first electronic absorption band of protonated adenine.

The most important question is how and by which tautomer(s) the measured broad spectrum of protonated adenine can be explained. In general there are three possibilities:

(a) The spectrum is composed of the spectra of several tautomers, thus causing a high line density.

(b) The geometry of the S$_1$ state is strongly shifted in comparison to the S$_0$ state geometry, allowing access only to high vibrational energy where the density of states is high.

(c) The lifetime of the S$_1$ state is very short, leading to a broadening of the spectral lines.

Case (a): We have no tautomer-selective measure and therefore we cannot exclude a superposition of several tautomer spectra from the experiment alone. As reported above, the calculations predict 1H-9H-A$^+$ to be the most stable tautomer in the gas phase. Protonation of 7H-A in N$_3$-position yields a tautomer (3H-7H-A$^+$), which is less stable by merely 162 cm$^{-1}$. The 3H-9H-A$^+$ tautomer already lies 688 cm$^{-1}$ above the most stable tautomer. Other tautomeric structures are located at markedly higher energies and are not considered in the following discussion.

Unfortunately we do not know the height of the barriers that separate the tautomers in gas phase. One can assume that they are high because two hydrogens have to be exchanged simultaneously. The details of the last stage of the desolvation in the ESI process, the transition from solution to the gas phase, are unknown. In clusters proton transfer is supposed to occur via proton wires for which typically two or three water molecules are required. Therefore two protons have to migrate to interconvert 1H-9H-A$^+$ and 3H-7H-A$^+$, the decision which above the electronic ground state at the same geometry. The population of this tautomer after photoexcitation could hence be detected by a strongly red-shifted emission. If the lifetime of the first electronically excited singlet state was long enough, the carbon atom in 2-position would represent the ideal proton acceptor site. The property of this site to act as a photo basic donor is very strong and the planarity is more or less conserved, we would supposedly see the origin of the $\pi n^*$ transition in the experimental spectrum.

The energy at the crossing is again very similar to the one found for 1H-9H-A$^+$ tautomer because its transition dipole is very strong and the planarity is more or less conserved, we would supposedly see the origin of the $\pi n^*$ transition in the experimental spectrum. Due to the conical intersections of the S$_1$ and S$_0$ PEHs of the most abundant tautomers (1H-9H-A$^+$, 3H-7H-A$^+$, 3H-9H-A$^+$) and the expected ultrafast S$_1$ relaxation the tautomerisation in the excited state involving a protic solvent should be of minor importance.
tautomer is formed in the desolvation process should take place at a cluster size of adenine with several water molecules.

Due to this, the equilibrium of the tautomers found in the gas phase after the ESI process might be close to the tautomer equilibrium in solution. Calculations of relative tautomer energies in a closed solvent shell with high accuracy are difficult. Binding energies of adenine with one water might be sufficient to show at least whether a strong solvent effect can be expected or not. 1H-9H-A\(^+\) and 3H-7H-A\(^+\) can form bridged hydrogen bonds with one water molecule which are substantially stronger than a single hydrogen bond (see section III). Nevertheless, the solvation by one water molecule further shifts the equilibrium toward the 1H-9H-A\(^+\) tautomer. The 3H-9H-A\(^+\) tautomer cannot form a geometrically favourable bridged structure with one water and therefore gains less relative energy by solvation in comparison to the 1H-9H-A\(^+\) and 3H-7H-A tautomers. The energetic order of the first three tautomers with one water is as following: 1H-9H-A\(^+\): 0 cm\(^{-1}\); 3H-7H-A\(^+\): 274 cm\(^{-1}\); 3H-9H-A\(^+\): 1545 cm\(^{-1}\).

Therefore, only the two nearly equally stable 3H-7H-A\(^+\) and 1H-9H-A\(^+\) tautomers are expected to be present in the beam after the ESI process. The tautomer equilibrium as formed in the ESI source is expected to be transferred to the Paul trap because in gas phase the barrier to move two hydrogens simultaneously is expected to be very high.

The calculations show that the \(S_0\)–\(S_1\) spectra of the 1H-9H-A\(^+\) and the 3H-7H-A\(^+\) tautomer should be shifted with respect to each other, with the spectrum of the 3H-7H-A\(^+\) lying at higher energy. So, the onset of the photofragment spectrum is expected to be very high.

As presented in section III, the quantum chemical calculations yield a non-planar structure in the first excited state of the two lowest-energetic tautomers. Thus, for both tautomers a situation as shown in Fig. 8a arises. The excited state has a double-well structure in an out-of-plane coordinate. In the corresponding vibrational mode the FC overlap gradually increases with excited state quantum number, reaches a maximum close to the barrier and then decreases above the barrier (see schematic spectrum in Fig. 8b). According to this situation, we expect a smooth onset (Fig. 8b) for both tautomers and a lifetime broadening (Fig. 8c) due to the conical intersection. Note that the reaction coordinate for the ring puckering is not a normal coordinate in the ground state and that therefore Fig. 8 can be only qualitative.

The remaining differences between the two lowest tautomers then are: (i) the depth of the double well, (ii) the absolute position of the first excited \(\pi^*\) state origin and (iii) the flat local minimum of the 3H-7H-A\(^+\) tautomer in the FC region on top of the barrier between the double well minima.

In the following the effects of the geometry shifts between \(S_0\) and \(S_1\) are discussed for the individual tautomers. For the 1H-9H-A\(^+\) tautomer our DFT/MRCI calculations place the planar \(\pi^*\) excited electronic structure is located beyond the intersection seam. In the conical intersection the puckering of the purine ring is very strong (see Fig. 5 right), the \(H_2-C_5-C_4-C_3\) dihedral angle being close to 90°. In total the 1H-9H-A\(^+\) tautomer has two equivalent minima below and above the planar geometry. Since the barrier height of the planar structure amounts to at least 0.5 eV (4000 cm\(^{-1}\)) and the vibrational frequency of the puckering mode is expected to be low, the double minimum potential (drawn schematically in Fig. 8a) can house several vibrational states below the barrier. Due to the strong \(S_0\)–\(S_1\) geometry change a direct optical access to the \(\pi^*\) origin is unlikely. As the vibrational levels approach the barrier, the overlaps increase. A spectral behaviour of this kind was observed earlier for a linear to bent transition in the acetylene radical cation.\(^{49}\) The broad structure in the optical spectrum of the 1H-9H-A\(^+\) tautomer is then attributed to the high density of the transitions to out-of-plane modes in combination with a lifetime broadening caused by ultrafast non-radiative decay through the conical intersection to the electronic ground state. Typically, the change of the electronic surfaces in conical intersections occurs on the femtosecond time scale, corresponding to line widths in the 100 cm\(^{-1}\) range. Because the quenching of the electronic excitation by the conical intersection is very efficient, the quantum yield for the following statistical dissociation out of vibrationally hot ground state levels is high.

---

*Fig. 8* (a) Schematic course of the ground and \(1\pi^*\) potential energies along the puckering coordinate in the 1H-9H-A\(^+\) tautomer. Vibrational levels and wave functions are not drawn to scale. Only symmetric vibrational states are sketched. The ground state vibrational wave function preferentially has a good overlap with excited state vibrational wave functions close to the barrier toward planarity. (b) Schematic absorption spectrum resulting for the potential energy surfaces on the left. (c) Expected spectral structure of (b) broadened by lifetime effects as caused by ultrafast nonradiative decay to the electronic ground state through the conical intersection. For further explanations see text.
In the FC region, the $S_1$ PEH of 3H-7H-A$^+$ exhibits a local minimum with an adiabatic DFT/MRCI excitation energy of 4.72 eV. The oscillator strength of the electronic transition from the ground state is large, about two times the value calculated for the $S_0 \rightarrow S_1$ transition in 1H-9H-A$^+$. Inspite of the presumably lower abundance in the molecular beam the 3H-7H-A$^+$ tautomer is therefore expected to be visible in the UV fragmentation spectrum. As for the 1H-9H-A$^+$ tautomer, the global minimum of the $S_0$ PEH of 3H-7H-A$^+$ is located at its conical intersection with the electronic ground state at a strongly puckered geometry, but the depth of the double-well potential (0.22 eV) is less than half the size of the one in 1H-9H-A$^+$. Because of the large geometry change, we nevertheless expect the onset of the 3H-7H-A$^+$ absorption spectrum to be smooth. However, the onset should be located at markedly higher energies than for the 1H-9H-A$^+$ tautomer since the conical intersection with the electronic ground state occurs at about 4.5 eV in 3H-7H-A$^+$ compared to about 4.1 eV in 1H-9H-A$^+$. The onset of our gas phase UV fragmentation spectrum at 4.35 eV is therefore not compatible with our quantum chemical results for the 3H-7H-A$^+$ tautomer.

Supposing that the above-described scenario is correct, how can we explain the vibronic structure appearing on top of the broad photo-fragmentation spectrum of protonated adenine (Fig. 2)? To this end, we assume that the stretching modes do not strongly couple with the puckering mode and can be considered as being nearly orthogonal. In this case, we can use the harmonic stretching frequencies and FC factors computed at the relaxed planar $^1\text{π}^\pi^*$ geometry to estimate the peak positions and intensities for the out-of-plane distorted structure. According to this model, the broad peak with a maximum at approximately 4.53 eV is interpreted as a series of broadened transitions to n, n + 1, n + 2, .... quantum of the puckering mode while all perpendicular stretching modes remain in the zeroth vibrational state. Correspondingly, the peak around 4.59 eV is assumed to arise from a transition to a level in which the in-plane vibrational mode with frequency near 500 cm$^{-1}$ is excited to its first vibrational state overlaid again by n, n + 1, n + 2, .... quantum of the puckering mode. Similar bands would be expected to emerge above 1000 and 1500 cm$^{-1}$ above the 4.53 eV peak, as our FC analysis at the saddle point has shown (see case b) that these vibronic excitations exhibit large FC factors.

(c) Ultrafast non-radiative decay of the vibronically excited states is expected to broaden the spectral lines due to the short lifetime. Thus a smooth rising and steeply falling broad structure is expected (see Fig. 8c). We are well aware that in the case of an ultrafast conical intersection the vibrational and electronic problem is strongly coupled leading to a more complex situation. Nevertheless, we believe that Fig. 8 qualitatively shows the right effect of Franck–Condon factors for this kind of an ultrafast conical transition.

From the above discussion it is clear that the lowest two tautomers have double well-minimum structures in the upper state that exhibit low-energetic conical intersections with the ground state. Therefore, they have presumably broad spectra and a short lifetime. The question is which of these tautomers is responsible for the soft onset of the experimental spectrum. The onset and the shape of the observed UV fragmentation spectrum of protonated adenine matches well with our theoretical results for the 1H-9H-A$^+$ tautomer. However, taking into account that the ZPVE correction typically lowers the excitation energy by some 100 cm$^{-1}$ and that our theoretical prediction is afflicted with an uncertainty, we cannot completely rule out the possibility that the spectrum originates from the second stable tautomer, 3H-7H-A$^+$. We consider this possibility to be highly improbable, though. For related compounds (such as 9H-adenine, 16 2-aminopurine, 67 cytosine, 58 as well as neutral and protonated tryptophan, 37 where the origins of the lowest-lying $^1\text{π}^\pi^*$ states are known from high-resolution molecular beam experiments), quantum chemical studies employing the same methods as used here gave adiabatic excitation energies with an accuracy better than 0.1 eV. Because of this expected accuracy it would be very difficult to explain why the onset of the 3H-7H-A$^+$ absorption spectrum should start already at about 4.35 eV when the conical intersection occurs at about 4.5 eV and even is not directly accessible in the absorption process.

We are therefore confident that the smooth onset of the UV photofragment spectrum (Fig. 2) arises from transitions of the 1H-9H-A$^+$ tautomer in the molecular beam whereas the vibronic structure could be due to either of the two tautomers. Further experimental investigations on protonated 9Me-A and protonated 7Me-A could help resolving the ambiguity of our spectral assignment because they would be able to discriminate between these isomers.

V. Summary and conclusions

Evidence from our combined experimental and theoretical study of protonated adenine suggests that the spectrum is due to 9H-adenine protonated at the N1 site. Its UV absorption spectrum is broad and exhibits a wide, smooth onset. This spectral behaviour is deduced to originate from a conical intersection between the first $^1\pi\pi^*$ excited singlet state and the electronic ground state that offers an ultrafast relaxation pathway for electronically excited 1H-9H-A$^+$ ions. As reaction coordinate we have identified the puckering of the six-membered purine ring. A similar relaxation pathway exists in neutral adenine. However, in neutral adenine the conical intersection is separated from the $^1\pi\pi^*$ minimum by a transition state. In molecular beam experiments of neutral 9H-adenine therefore sharp vibronic transitions close to the origin can be measured. This is different in the protonated form. In the Franck–Condon region, no minimum has been detected on the $^1\pi\pi^*$ potential energy surface. Rather, the conical intersection with the electronic ground state can be reached after electronic excitation without the necessity to overcome a barrier. As described above, the relaxation coordinate is not dissociative. From the aspect of photostability, protonated adenine is thus superior to the neutral form. In the adenine-thymine Watson–Crick base pair, the N1 atom of adenine acts as a hydrogen-bond acceptor. The hydrogen-bonded situation can be seen as an intermediate situation between a protonation and a free electron-pair at the position N1. It can therefore be supposed that the puckering of the six-membered ring is eased by the hydrogen bonding and the concomitant ultrafast relaxation pathway is readily accessible in the Watson–Crick base pair.

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