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IR–UV double-resonance spectroscopy of the nucleobase adenine

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We present R2PI and IR–UV double resonance spectra of the nucleobase adenine seeded in a supersonic jet. We show that there is only one tautomer of adenine which absorbs in the wavelength range 36 050 to 36 700 cm^{-1}. The IR spectra, measured in the range 3200 to 3700 cm^{-1}, show bands at 3452, 3508 and 3569 cm^{-1}, which we assign to the symmetric NH_2, N–H and antisymmetric NH_2 stretching vibrations of a single tautomer of adenine. We compare the experimental IR–UV double resonance spectra with *ab initio* based normal mode calculations. The observed tautomer is most probably the 9H amino-form of adenine.

1. Introduction

Understanding of the photochemistry and photophysics of the DNA bases is important because of the mutagenic and carcinogenic potential of UV absorption in nature.[1,2] In a previous publication Nir *et al*. reported the electronic spectra of a number of purine nucleobases and their derivatives by using laser desorption combined with resonance-enhanced two-photon ionisation (R2PI) spectroscopy.[3] For adenine they recorded only four bands in the range 36 000 to 36 500 cm^{-1}. Kim *et al*. investigated adenine with one- and two-color R2PI and laser induced fluorescence (LIF).[4] Their LIF spectrum is in good agreement with the R2PI spectrum of Nir *et al*.[3] Their R2PI spectrum shows more bands in the ranges 35 400 to 36 000 cm^{-1} and 36 400 to 37 000 cm^{-1}. Bands between 35 400 and 36 000 cm^{-1} could only be observed by strongly focusing the UV-laser. Kim *et al*. postulate that two different electronic transitions may contribute to the spectrum, namely a $n\pi^*$ transition for the red part and a $\pi\pi^*$ transition for the blue part. They also discuss a possible coupling between these two states. Lührs *et al*.[5] observed only transitions of adenine in the range 36 000 to 36 700 cm^{-1} and a weak band at 35 822 cm^{-1} using R2PI for detection.

Only the 9H amino-tautomer was observed by microwave spectroscopy in a supersonic beam experiment.[6] Bernath and coworkers published IR gas cell spectra in the range 100 to 3700 cm^{-1}.[7] Matrix isolation spectra in rare gases and *ab initio* calculations show vibrations in the same range.[8] Previous observations of Janzen *et al*.[9] with a combination of GC and IR spectroscopy show vibrations in the same range as described by Colarusso *et al*.[7]

In this paper we report the electronic spectra of the DNA base adenine as well as its ground state IR spectrum in the region of the NH-vibrations. We use R2PI to investigate the excited state and IR–UV double-resonance spectroscopy to obtain tautomer selected IR spectra. We compare the experimental results with the results of *ab initio* based normal mode calculations at the MP2/6-311G(d, p) and B3LYP/6-311G (d, p) level of theory. Adenine can adopt 8 different tautomeric forms.[9] Results from several calculations show that the 9H amino-tautomer has the lowest stabilization energy followed by the 7H amino-tautomer and the 9H/7H imino-forms.[10–12] The structures of the different tautomers are shown in Fig. 1.

2. Experimental and theoretical methods

The apparatus used for IR–UV double-resonance spectroscopy has been described in detail elsewhere.[13] Briefly, it consists of a source chamber pumped with a 3000 l s^{-1} oil diffusion pump (Leybold) in which the molecular beam is formed by expanding a mixture of helium and adenine at 210–240 °C through the 300 μm orifice of a pulsed nozzle (General Valve, Iota One). The laser beams cross the skimmed molecular beam (Beam Dynamics Skimmer, 1 mm orifice) at right angles in the ionization chamber. The ions are extracted in a modified Wiley–McLaren type time-of-flight (TOF) spectrometer perpendicular to the molecular and laser beams and enter the third (drift) chamber, where they are detected using

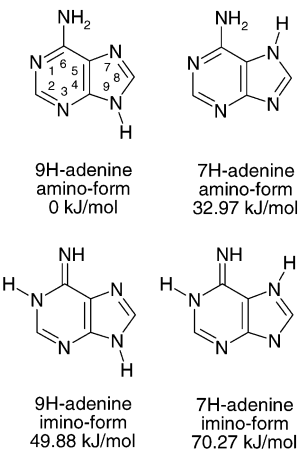

Fig. 1 Structures of different tautomers of adenine. The 9H-tautomer is the most stable tautomer. See text and Table 1 for the calculated relative energies of the different tautomers.

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multi-channel plates (Topag). Typical mass resolution of the spectrometer is $m/\Delta m = 500$.

The R2PI measurements were carried out using the frequency doubled output of a Nd:YAG (Spectra Physics, GCR3) pumped dye laser (LAS, LDL205) operated with Rhodamine 6G and Fluorescein 27. The dye laser was calibrated by recording an iodine vapor spectrum and comparison with its tabulated transition frequencies. The IR light (3000–4000 cm^{-1}) was generated with a $LiNbO_3$ crystal by difference frequency mixing of the fundamental (1064 nm) of a seeded Nd:YAG laser (Spectra-Physics Quanta Ray Pro 230) and the output (745–800 nm) of a dye laser (Sirah Precision Scan D) pumped by the second harmonic (532 nm) of the same Nd:YAG laser. The IR laser was calibrated by recording a water vapor spectrum and comparison with its tabulated transition frequencies.

In addition to the molecular beam experiments, the IR spectroscopy of the adenine monomer was also performed[9] using a Hewlett Packard system consisting of a GC 5890 Series II gas chromatograph, a Fourier transform infrared spectrometer (IRD 5965 B), equipped with a wide-band detector with a frequency range between 550 and 4000 cm^{-1}, and finally a mass detector (MSD 5971). Adenine has to be heated to around 280 °C to obtain a vapor pressure sufficient for IR spectroscopy, however at this temperature it decomposes to some extent. Hence an infrared spectrum obtained in a simple heated cell consists of IR bands of adenine and decomposition products.[9] GC/FTIR/MS has the advantage that the intact adenine and decomposition or reaction products of adenine are gas chromatographically separated and that for each gas chromatographic peak an IR spectrum and a directly correlated mass spectrum can be taken within a 15–20 s delay time. With this method we were able to get the IR spectrum of the adenine monomer unambiguously identified by retention time and mass spectrum.[9]

All *ab initio* calculations were performed using the Gaussian 98 program package.[14] The SCF energy limit used in the convergence criterion was 1×10^{-8} E_h and the convergence criterion for the gradient optimization of the molecular geometries was 1.5×10^{-5} E_h a_0^{-1} and E_h (degree)$^{-1}$, respectively.

The geometry optimization of the different tautomers was performed by using Møller–Plesset pertubation theory at the second order (MP2) level with the 6-311G(d, p) basis set. We also used density functional theory (DFT) with the same basis set and the B3LYP functional which is provided by the Gaussian 98 program. The vibrational frequencies of the ground state were calculated using the analytical second derivatives of the MP2 and the B3LYP potential energy surfaces.

3. Results and discussion

Fig. 2 shows the one-color, two-photon ionization spectrum of adenine in the spectral range 35 800 to 36 700 cm^{-1}. The details of the vibronic spectrum were discussed elsewhere.[3] The small band at 35 824 cm^{-1} was only observed when the ionization laser was strongly focused. This band is in a spectral range where Kim *et al.*[4] attributed some small bands to the nπ* transition of adenine. Unfortunately the band was too weak to perform IR–UV double resonance experiments. Many fragments of adenine were formed, when focusing the R2PI laser. Therefore it is possible that the bands observed under these conditions are due to three or more photon absorption of adenine or adenine-clusters. We measured the same vibronic frequencies of adenine as given in ref. 5 and therefore we do not publish them here in a separate table.

Fig. 3 displays the IR spectrum of adenine taken with the UV analysis laser tuned to 36 104.6 cm^{-1}. The IR spectrum taken in the range 3200–3700 cm^{-1} shows bands at 3452, 3508 and 3569 cm^{-1} which can be assigned to the symmetric NH_2, N–H and antisymmetric NH_2 stretching vibrations of adenine. The other vibronic bands in Fig. 2 exhibit IR spectra with exactly the same IR-frequencies and relative intensities. Therefore we conclude that we observe only one tautomer of adenine in the spectral range displayed in Fig. 2. The IR spectra taken in a heated cell[7] and with GC/FTIR/MS[9] show bands at 3434[7] and 3436[9] cm^{-1}, 3501[7] and 3498[9] cm^{-1}, 3552[7] and 3547[9] cm^{-1}, respectively. However these spectra are not tautomer selected. They are taken at temperatures of 280–325 °C. Matrix-isolated IR-spectra show bands at 3441/3448 cm^{-1}, 3489/3498 cm^{-1} and 3557/3565 cm^{-1}.[8] The observed splittings originate from interactions with the matrix. We note that the gas cell IR bands are similar to the red component and our results in the supersonic jet to the blue component of the split matrix bands.

The calculated stabilization energies of the different tautomers of adenine are presented in Table 1. The most stable

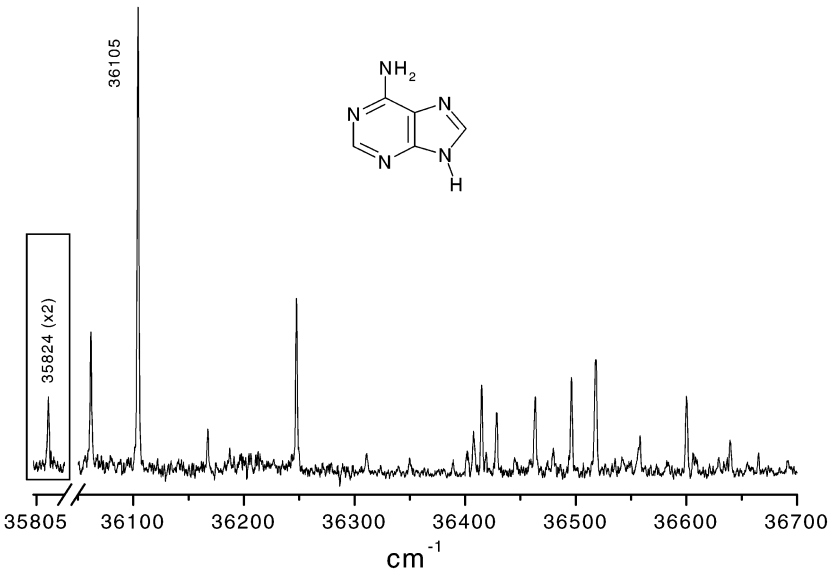

Fig. 2 R2PI spectrum of adenine in a supersonic jet after evaporation of adenine at 220 °C. The inset shows a very weak band observed at 35 824 cm^{-1}. This band was only observed when the R2PI laser was focused.

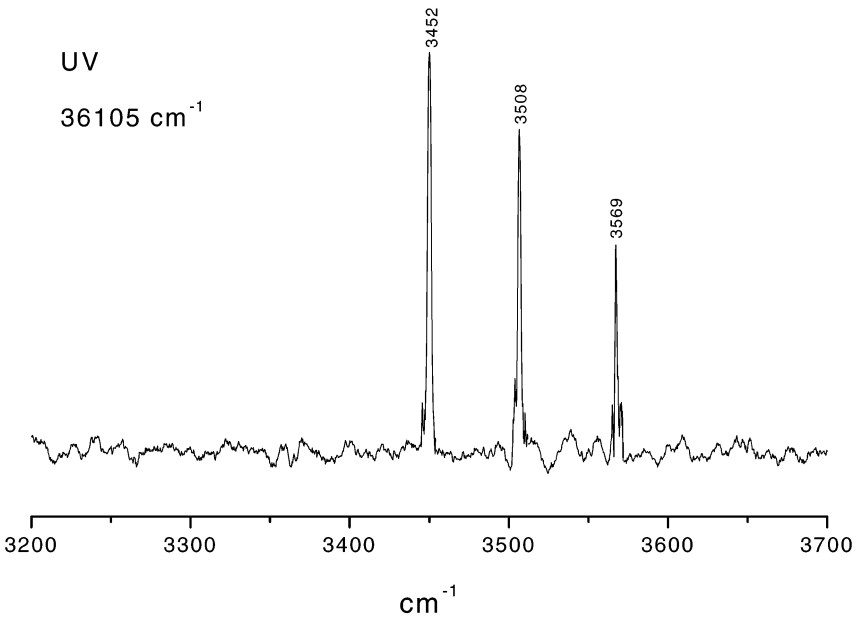

Fig. 3 IR–UV double resonance spectrum of adenine with the R2PI laser set to 36 105 cm^{-1}. The bands at 3452, 3508 and 3569 cm^{-1} are the symmetric NH_2, N–H and antisymmetric NH_2 stretching vibrations of adenine, respectively.

Table 1 Stabilization energies of the different tautomers of adenine. The MP2 and the B3LYP calculations have been performed with the split valence 6-311G(d,p) basis set

	Energy/kJ mol^{-1}	
Tautomer	MP2	B3LYP
9H-Adenine	0	0
7H-Adenine	+33.0	+36.0
9H-Imino	+49.9	+48.8
7H-Imino	+70.3	+69.3

tautomer is the 9H amino-tautomer, followed by the 7H amino-tautomer which is 36.0 kJ mol^{-1} higher in energy. The stabilization energy for the 9H imino-form is 48.8 kJ mol^{-1} and for the 7H imino-form 69.3 kJ mol^{-1} higher than the 9H amino-tautomer at the same level of theory. The calculated frequencies at B3LYP/6-311G(d, p) and MP2/6-311G(d, p) presented in Table 2 and Table 3 are in good agreement with the experimental results from the IR–UV double resonance spectrum for the 9H amino-tautomer. The shifts of the symmetric NH_2-, the NH- and the antisymmetric NH_2-vibrations for the 9H amino-tautomer at the MP2/6-311G(d, p) level are −1.4, −1.1 and −1.1%, respectively. The shifts of the calculated 7H amino-tautomer frequencies are −2.4, −1.0 and −2.6%, *cf*. Table 2. For the imino-forms the deviations are even larger, *cf*. Table 3. We conclude that we most probably observe the 9H amino-tautomer in the supersonic jet. From the comparison of experimental and theoretical results we conclude that the gas cell results, without the jet cooling, may consist of spectra from a mixture of 9H/7H amino-tautomers and possibly the imino-forms at the cell temperature.

Table 2 Experimental IR frequencies together with the calculated vibrational frequencies for the amino forms of adenine in the region of the NH vibrations. All calculations have been performed at the MP2/6-311G(d, p) and B3LYP/6-311G(d, p) level of theory. All frequencies have been scaled and given in cm^{-1}. MP2 scaling factor: 0.9427; B3LYP scaling factor: 0.9613 both as recommended by ref. 15 for the 6-31G(d) basis set

		9H-Adenine				7H-Adenine			
Assignment	Experiment	MP2	Δ (%)	B3LYP	Δ (%)	MP2	Δ (%)	B3LYP	Δ (%)
sym NH_2	3452	3405	−1.4	3467	+0.4	3368	−2.4	3419	−1.0
NH	3508	3471	−1.1	3510	+0.1	3473	−1.0	3514	+0.2
antisym NH_2	3569	3529	−1.1	3595	+0.7	3475	−2.6	3524	−1.3

Table 3 Experimental IR frequencies together with the calculated vibrational frequencies for the imino forms of adenine in the region of the NH vibrations. All calculations have been performed at the MP2/6-311G(d, p) and B3LYP/6-311G(d, p) level of theory. All frequencies have been scaled and given in cm^{-1}. MP2 scaling factor: 0.9427; B3LYP scaling factor: 0.9613 both as recommended by ref. 15 for the 6-31G(d) basis set

		9H-Imino				7H-Imino			
Assignment	Experiment	MP2	Δ (%)	B3LYP	Δ (%)	MP2	Δ (%)	B3LYP	Δ (%)
Imino NH	3452	3330	−3.5	3368	−2.4	3311	−4.1	3346	−3.1
1NH	3508	3420	−2.5	3456	−1.5	3428	−2.3	3466	−1.2
9/7NH	3569	3466	−2.9	3506	−1.8	3460	−3.0	3504	−1.8

4. Summary

IR–UV hole burning spectroscopy of adenine proves that the jet-cooled vibronic spectrum between 36 050 and 36 700 cm^{-1} consists of a single tautomer. Comparison with *ab initio* calculations suggests that this is the 9H form of adenine. Without the jet-cooling the IR spectrum of adenine is more complex, indicating a tautomeric mixture. We cannot exclude that other tautomers exist in the supersonic jet which do not absorb in the investigated wavelength range.

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