

Pairing of Isolated Nucleobases: Double Resonance Laser Spectroscopy of Adenine – Thymine

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The vibronic spectrum of the adenine–thymine (A–T) base pair was obtained by one-color resonant two-photon ionization (R2PI) spectroscopy in a free jet of thermally evaporated A and T under conditions favorable for formation of small clusters. The onset of the spectrum at 35 064 cm⁻¹ exhibits a large red shift relative to the π – π^ origin of 9H-adenine at 36 105 cm⁻¹. The IR–UV spectrum was assigned to cluster structures with HNH...O=C/N...HN hydrogen bonding by comparison with the IR spectra of A and T monomers and with ab initio calculated vibrational spectra of the*

most stable A–T isomers. The Watson–Crick A–T base pair is not the most stable base-pair structure at different levels of ab initio theory, and its vibrational spectrum is not in agreement with the observed experimental spectrum. Experiments with methylated A and T were performed to further support the structural assignment.

KEYWORDS:

ab initio calculations · IR spectroscopy · laser spectroscopy · nucleobases · UV/Vis spectroscopy

Introduction

High-resolution IR and UV spectroscopy on isolated biomolecular building blocks can reveal intrinsic molecular properties such as tautomerism, conformer folding, electronic and vibrational states, and cluster formation in great detail. Here, we report the first electronic and IR spectra of the isolated adenine–thymine base pair. Several groups were able to record the electronic spectra of the adenine monomer by means of resonant two-photon ionization (R2PI)^[1–3] and laser-induced fluorescence (LIF)^[1] and observed the origin of the π – π^* transition at 36 105 cm⁻¹. Only the 9H-amino tautomer was observed with microwave spectroscopy in a supersonic beam.^[4] Bernath and co-workers published gas-cell IR spectra of adenine in the range of 100–3700 cm⁻¹.^[5] Matrix-isolation spectra in noble gases and ab initio calculations show vibrations in the same range.^[6] Previous investigations on adenine by us^[7] with GC-MS and IR spectroscopy showed an IR spectrum similar to that described by Colarusso et al.^[5] Recent tautomer-selected IR-UV double-resonance spectra of adenine thermally evaporated in a jet revealed that only the 9H tautomer of adenine absorbs in the wavelength range of 36 050–36 700 cm⁻¹,^[8] while 7H-adenine is a minor tautomer with a very different IR spectrum and a UV absorption at longer wavelengths than 9H-adenine.^[9]

The electronic spectrum of thymine is broad and structureless^[10] with an onset at about 35 800 cm⁻¹. Vibrational spectra of thymine were obtained by FTIR^[5] and GC-FTIR-MS^[7] spectroscopy. The IR spectra of the monomeric bases are valuable guides for the assignment of the spectrum of the A–T clusters to a specific structure, as will be shown in the following.

Numerous theoretical studies have been performed on A–T clusters. In particular, Hobza and co-workers carried out

extensive quantum chemical and molecular dynamics calculations on hydrogen-bonded and stacked DNA base pairs and their methylated derivatives.^[11]

Experimental Section

The apparatus used for IR-UV double-resonance spectroscopy has been described in detail elsewhere.^[12] 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, adenine, and thymine at 210–260 °C through the 500 μ m orifice of a pulsed nozzle (General Valve, Iota One) equipped with Kalrez O-rings resistant to high temperatures. The nozzle was heated to 300 °C. Adenine and thymine were stored in small glass containers in two separately heated steel chambers. This arrangement serves to reduce thermal decomposition and to adjust optimum vapor pressures for pair formation. The relative timing of the gas pulse and the laser trigger also proved to be important for obtaining R2PI spectra of A–T, since conditions must be optimized for formation of small clusters.

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The laser beams cross the skimmed molecular beam (Beam Dynamics Skimmer, 2 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 by a multichannel plate detector (Topag). Typical mass resolution of the mass spectrometer is $m/\Delta m = 500$.

The R2PI measurements were carried out with the frequency doubled output of an Nd:YAG (Spectra Physics, GCR3) pumped dye laser (LAS, LDL205) operated with Rhodamine 6G and Fluorescein 27. The dye laser was calibrated by recording the spectrum of iodine vapor and comparing it with tabulated transition frequencies.^[13] The IR radiation ($3000\text{--}4000\text{ cm}^{-1}$) was generated with a LiNbO₃ 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 fired 50–100 ns prior to the UV laser. The IR laser was calibrated by recording a spectrum of water vapor and comparing it with tabulated transition frequencies.^[14]

In addition to the molecular beam experiments, conventional gas-phase IR spectroscopy of the adenine and thymine monomers was performed on a Hewlett Packard system consisting of a GC 5890 Series II gas chromatograph, a Fourier transform IR spectrometer (IRD 5965 B) equipped with a wide-band detector with a frequency range of $550\text{--}4000\text{ cm}^{-1}$, and a mass detector (MSD 5971).^[7] Adenine and thymine must be heated to around $280\text{ }^{\circ}\text{C}$ to obtain a vapor pressure sufficient for IR spectroscopy, but at this temperature they decompose to some extent. Hence an infrared spectrum obtained in a simple heated cell consists of IR bands of adenine or thymine and decomposition products.^[7] GC-FTIR-MS has the advantage that the intact monomers and their decomposition or reaction products are separated by gas chromatography and that for each gas-chromatographic peak an IR spectrum and a directly correlated mass spectrum can be recorded within a time delay of 15–20 s. With this method we were able to record the IR spectrum of the adenine and thymine monomers, unambiguously identified by the retention time and the mass spectrum.^[7]

Adenine (99.5%, Acros Organics), 9-methyladenine (98%, Acros Organics), thymine (>98%, Merck), and 1-methylthymine (>99%, Sigma) were used without further purification. 7-Methyladenine (Acros Organics) was specified with a purity >90% and contains 9-methyladenine according to its REMPI (resonance enhanced multiphoton ionisation) spectrum.

All ab initio calculations were performed with the Gaussian98 program package^[15] at the HF/6-31G(d,p) level. The SCF energy limit used in the convergence criterion was $1 \times 10^{-8} E_h$, and the convergence criteria for the gradient optimization of the molecular geometries were $1.5 \times 10^{-5} E_h a_0^{-1}$ and $E_h(\text{deg})^{-1}$. The vibrational frequencies were obtained by performing a normal-mode analysis on the optimized geometries using analytical gradients of the energy. The stabilization energies D_e were corrected for the zero-point energy (ZPE) by using the harmonic frequencies.

Results and Discussion

Figure 1 shows the TOF mass spectra of thermally evaporated adenine and thymine in a free jet. Figure 1a shows the mass spectrum with the ionization laser triggered at the peak of the jet gas pulse. Figure 1b displays the mass spectrum 100 μs earlier, at the onset of the plateau of the gas pulse, and

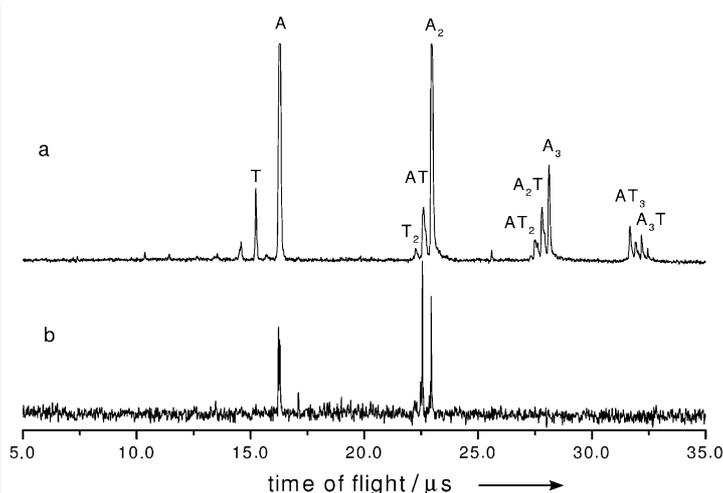


Figure 1. Time-of-flight mass spectra of adenine and thymine thermally evaporated in a free jet at a) the peak of the jet gas pulse and b) 100 μs earlier in the gas pulse. The width of the mass peaks in a) is broader than in b), probably because these ions arise from metastable decay of larger clusters (see text).

demonstrates that larger clusters ($> A_2$) are absent and are only formed later in the gas pulse. R2PI spectra with sharp spectral features could only be measured early in the gas pulse under conditions of Figure 1b. TOF mass spectra recorded later in the gas pulse showed a large fraction of protonated clusters $A_m T_n H^+$, which result from proton transfer upon laser excitation of larger clusters, followed by fragmentation. Our experiments showed that formation of larger clusters must be avoided to obtain sharp vibronic spectra of A–T with one-color R2PI.

Figure 2 shows the R2PI spectrum of A–T taken at its parent mass. The spectrum exhibits a number of sharp vibronic features built upon the reddest band at 35064 cm^{-1} . The excited moiety

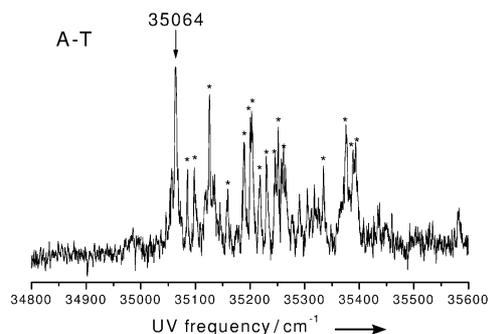


Figure 2. One-color R2PI spectrum of the adenine–thymine base pair taken under the conditions of Figure 1b with the mass gate set on the A–T parent mass. The arrow indicates the band used to record the IR spectrum of Figure 3. All bands marked with an asterisk were analyzed by IR-UV spectroscopy and show the IR spectrum displayed in Figure 3.

of the cluster is probably adenine, because thymine shows only a very broad unstructured spectrum with an onset at around 35800 cm^{-1} . The $\pi\text{--}\pi^*$ origin of the dominant tautomer 9H-adenine is at 36105 cm^{-1} , which corresponds to a large red shift of 1045 cm^{-1} and a correspondingly large cluster stabilization on electronic excitation. 9-Methyladenine–thymine (9MA–T)

shows a similar red shift of its R2PI spectrum relative to the 9MA monomer to A–T (see below), so that we can assume that 9H-adenine is the chromophore in the A–T cluster and not 7H-adenine. We measured a two-color ionization threshold of 69339 cm^{-1} for 9H-adenine (not field-corrected), so the band at 35064 cm^{-1} is possibly not the origin of the A–T spectrum but a vibronic band with just sufficient energy for two-photon ionization. Two-color ionization at 266 nm only increased the ion signal of the adenine monomer but not that of the A–T pair. Adenine shows a very broad, intense absorption band in this wavelength region^[1] and is therefore efficiently excited and ionized by two 266 nm photons. The A–T ion signal was diminished rather than enhanced by 35064 cm^{-1} excitation plus 266 nm ionization. We will perform two-color ionization at longer ionization wavelengths to check whether the origin of the A–T spectrum lies still further to the red.

The IR-UV spectrum of A–T taken with the UV probe at 35064 cm^{-1} is displayed in Figure 3. We performed IR-UV spectroscopy on all vibronic bands marked with an asterisk in Figure 2. All bands showed the same IR spectrum as that displayed in Figure 3 within $\pm 1\text{ cm}^{-1}$ experimental uncertainty and hence belong to the same A–T isomer. The spectrum shows six IR bands in the investigated spectral range of $3200\text{--}3650\text{ cm}^{-1}$. The IR spectra of thymine and 9H-adenine are shown for comparison. The assignment of the bands is based on comparison with frequencies calculated at the MP2/6-311G(d,p) level.^[8] The assignments for thymine, especially the order of the C=O and NH stretching vibrations, agree with those from ref. [5]. Additionally, we measured the FTIR spectrum of 1-methylthymine and observed only the band at 3435 cm^{-1} in the region of

NH stretching vibrations.^[16] Hence, the assignments of the 3435 cm^{-1} band to the N3H vibration and the 3481 cm^{-1} band to the N1H vibration are correct. The A–T band at 3437 cm^{-1} has a very similar frequency to the free N3H stretching vibration of the thymine monomer and is therefore not involved in hydrogen bonding. The doublet at $3437/3447\text{ cm}^{-1}$ probably arises from a Fermi resonance of the N3H stretching vibration of the thymine monomer with the first overtone of its C4=O stretching vibration at 1720 cm^{-1} . The free NH₂ stretching vibrations of 9H/7H-adenine at $3569/3550$ and $3452/3437\text{ cm}^{-1}$ are absent in the A–T spectrum. Hence, we conclude that the NH₂ group of adenine in A–T is involved in a hydrogen bond. Indeed we observe typical IR bands for a hydrogen-bonded NH₂ group at 3530 cm^{-1} (antisymmetric stretch) and 3326 or 3295 cm^{-1} (symmetric stretch). For comparison, the NH₂ antisymmetric stretching vibrations HNH...O=C of cytosine and guanine in cytosine–cytosine, guanine–guanine, and guanine–cytosine clusters are at 3525 , 3530 , and 3532 cm^{-1} , respectively. The frequency of the symmetric NH₂ stretching vibrations depends sensitively on the hydrogen-bond strength in these clusters and can shift far below 3300 cm^{-1} (see ref. [2] and Figure 4). Comparison of the band at 3507 cm^{-1} with the spectrum of 9H-adenine monomer shows that the N9H stretching vibration of adenine is free (not involved in hydrogen bonding). The bands at 3326 and 3295 cm^{-1} are the hydrogen-bonded NH₂ symmetric stretching vibration of adenine and the N1H stretching vibration of thymine, respectively. Comparison with the spectra of the monomers therefore allows us to conclude that the NH₂ group of adenine and the N1H group of thymine are involved in hydrogen bonding, while the N9H bond of adenine and the N3H bond of thymine are free.

Thus, we obtain initial information on possible cluster structures without performing calculations.

Figure 4 shows the experimental IR-UV spectrum of A–T and, for comparison, the structures, vibrational spectra, and relative stabilities of the doubly hydrogen bonded A–T isomers, calculated at the HF/6-31G(d,p) level. The two isomers whose calculated IR spectra best fit the experimental spectrum are marked by arrows. From the IR spectra alone we cannot differentiate between these two structures. The Watson–Crick A–T base pair (Figure 4g) is neither the most stable base pair at this level of theory nor at higher (correlated) levels,^[11] and its vibrational spectrum is not in agreement with the experimental spectrum. The spectra of the two most stable clusters at this level of theory (Figure 4a and b) also do not fit the observed IR spectrum. Both isomers have vibrations for free NH₂ groups which are clearly absent in the experimental IR spectrum of A–T.

For further structural discrimination we performed “blocking” experiments with A

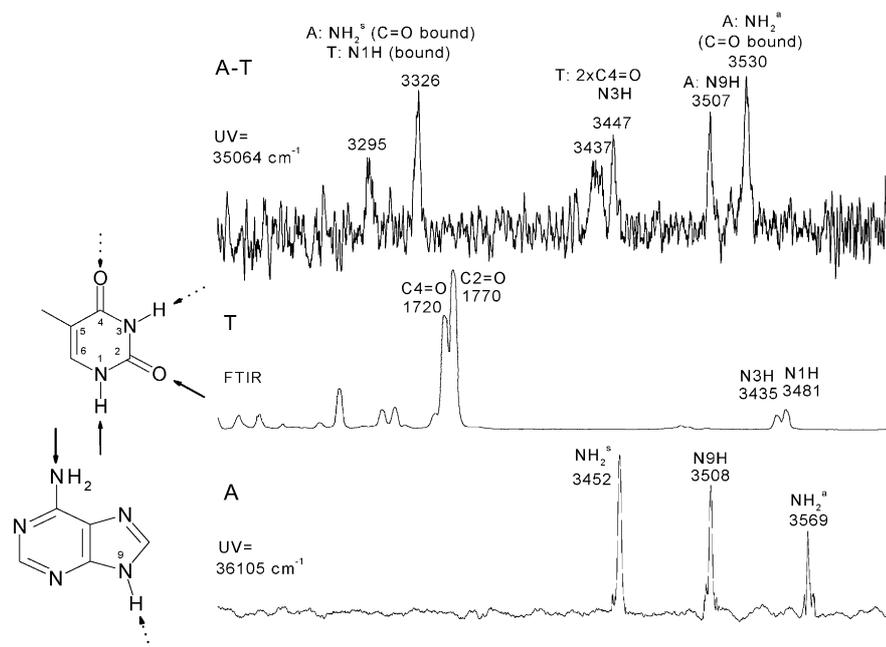


Figure 3. IR-UV double-resonance spectrum of the nucleobase pair A–T taken at 35064 cm^{-1} (upper trace). For comparison the FTIR spectrum of thymine (T) and the IR-UV spectrum of 9H-adenine (A) recorded at 36105 cm^{-1} are also shown. The thymine spectrum includes C=O vibration bands and is therefore displayed with a different frequency scale. Functional groups involved in hydrogen bonds are marked with full arrows, and “free” NH and C=O groups are marked with dotted arrows.

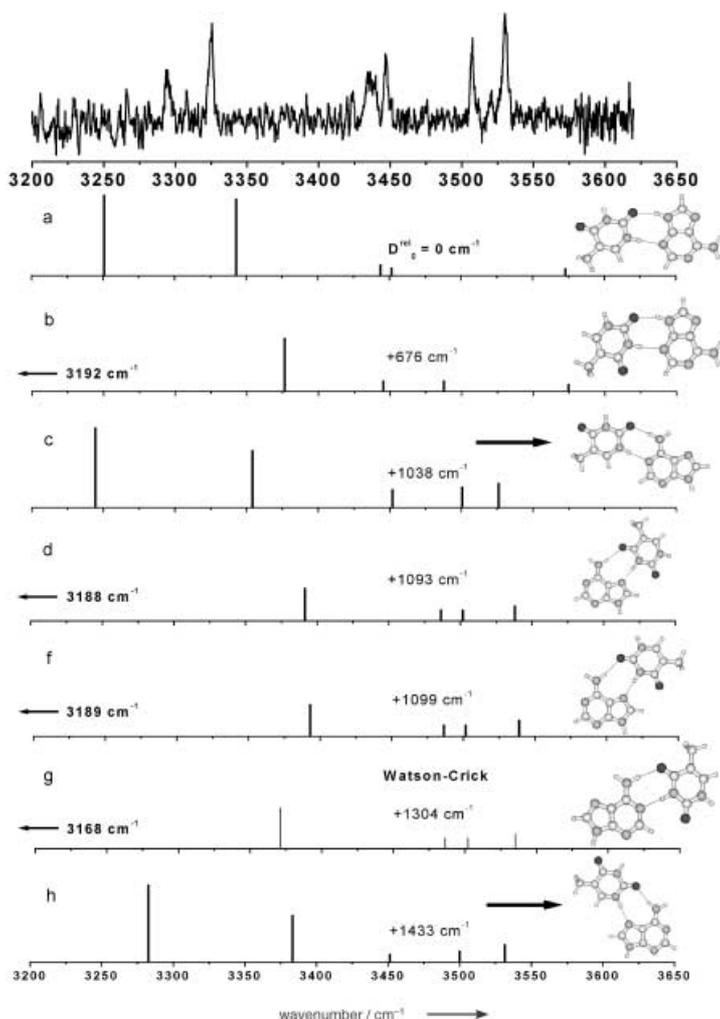


Figure 4. IR-UV spectrum of the nucleobase pair A–T and structures of the doubly hydrogen bonded A–T isomers (a–h). Their vibrational spectra and relative stabilization energies D_0 were calculated at the HF/6-31G(d,p) level and shown for comparison with the experimental spectrum. All frequencies were scaled by the factor 0.893, as used in our work on guanine–cytosine base pairs.^[17] The two isomers whose calculated IR spectra fit the experimental spectrum best are marked by arrows. The Watson–Crick A–T base pair is not the most stable base pair,^[11] and its vibrational spectrum is not in agreement with the experimental spectrum.

and T methylated in selected positions. Figure 5 shows the R2PI spectrum of 9MA–T, which exhibits a red shift relative to the 9MA monomer similar to that of A–T relative to 9H-adenine. This indicates that 9H-adenine is the chromophore in the A–T spectrum in Figure 2. We obtained the best quality of the 9MA–T spectrum by using thymine and 7MA containing 9MA as impurity as precursors (see Experimental Section). Again the spectrum in Figure 5 may not show the origin of the 9MA–T spectrum but possibly only vibronic bands with sufficient energy for two-photon, one-color ionization. The ionization potential of 9MA is around 0.1 eV lower than that of A.^[18] The R2PI spectrum of 9MA–T does not resemble that of A–T very closely, and shows quite harmonic progressions with a spacing of around 13 cm^{-1} . This low frequency is typical for an intermolecular vibration of the cluster, such as a butterfly motion or twisting of the cluster moieties. The UV spectrum of A–T in Figure 2 is much

less regular and shows spacings of 12 cm^{-1} for some bands. Figures 6 and 7 compare the IR-UV spectrum of 9MA–T with those of the monomer and with the calculated spectra of isomers c and h. Again the comparison shows that we cannot differentiate between isomers c and h. The frequencies of the hydrogen-bonded NH_2^s and N1H vibrations are not described well at the HF level of theory.

In adenine–1-methylthymine (1MT) formation of the most stable calculated cluster a and the two proposed isomers c and h is blocked, but the Watson–Crick structure g and stacked structures^[11] are possible. No electronic spectrum could be found for the A–1MT parent mass in the investigated range of $34800\text{--}36600\text{ cm}^{-1}$. This finding is in agreement with the two proposed structures but does not allow differentiation between them.

7-Methyladenine (7MA)–thymine can form isomer c, while structure h is blocked. Hence, this pair allows us to differentiate between the two proposed structures. We only observed the 9MA–T spectrum arising from the 9MA impurity in 7MA (see Figure 5), but no additional bands in the investigated range of $34800\text{--}36600\text{ cm}^{-1}$. However, this is not conclusive evidence for structure h, because 7MA is probably the chromophore in this wavelength range and its electronic spectrum could not yet be obtained.^[9] Possible reasons for the failure to detect the UV spectrum of 7MA–T are low thermal stability of 7MA, chemical reaction of 7MA with T in the stagnation chamber- and/or a red-shifted electronic absorption spectrum and a higher ionization potential that does not allow one color 1 + 1 ionization.^[9]

Conclusion

The IR-UV spectrum of A–T fits two cluster structures with $\text{HNH}\cdots\text{O}=\text{C}/\text{N}\cdots\text{HN}$ hydrogen bonding, based on comparison with the IR spectra of A and T monomers and with ab initio

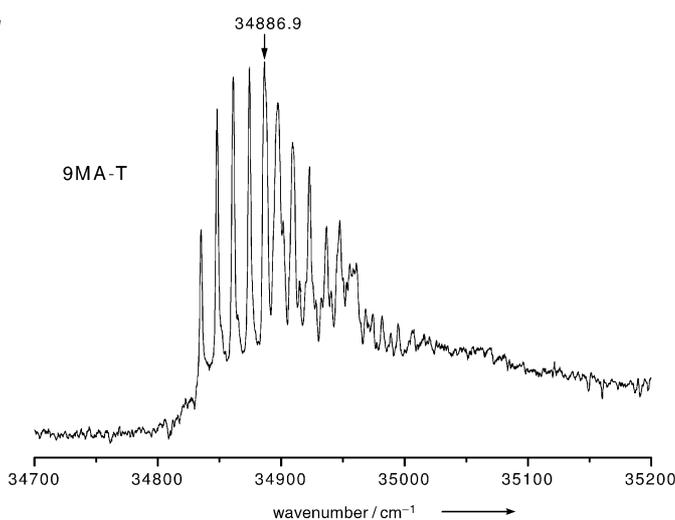


Figure 5. One-color R2PI spectrum of the 9MA–T base pair taken under the conditions of Figure 1 b with the mass gate set on the 9MA–T parent mass. The arrow indicates the band used to take the IR bands shown in the inset.

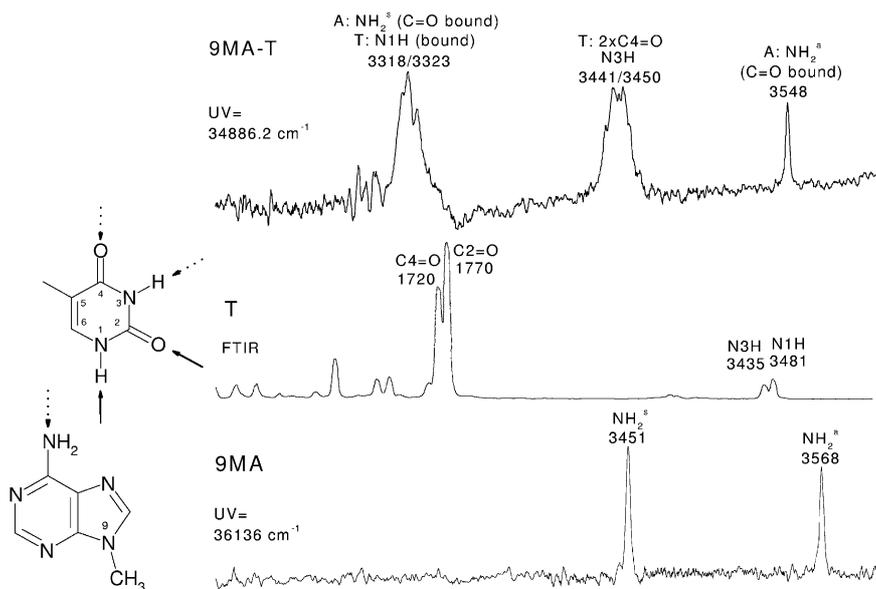


Figure 6. The IR-UV double resonance spectrum of the nucleobase pair 9MA–T taken at 34886 cm^{-1} (upper trace). For comparison the FTIR spectrum of thymine (T) and the IR-UV spectrum of 9-methyladenine (9MA) taken at 36136 cm^{-1} are also shown. Functional groups involved in hydrogen bonds are marked with full arrows, and “free” NH and C=O groups with dotted arrows.

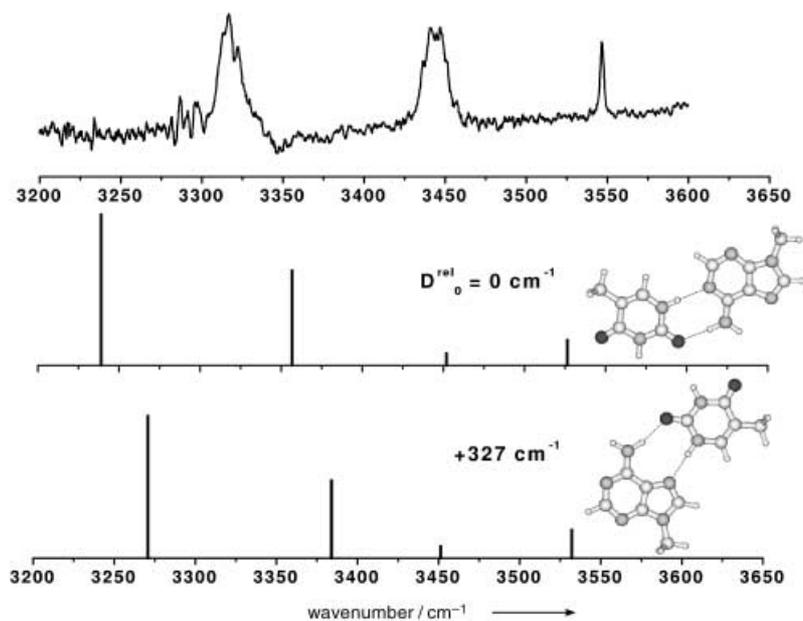


Figure 7. IR-UV spectrum of the nucleobase pair 9MA–T and structures of two doubly hydrogen bonded A–T isomers. Their vibrational spectra and relative stabilization energies D_0 were calculated at the HF/6-31G(d,p) level and are shown for comparison with the experimental spectrum. All frequencies were scaled by the factor 0.893, as used in our work on guanine–cytosine base pairs.^[17]

calculated vibrational spectra of the most stable A–T isomers. The Watson–Crick A–T base pair is not the most stable isolated A–T pair, and its vibrational spectrum is not in agreement with the observed experimental spectrum. This is directly shown by the free N3H vibration in the IR spectrum of A–T. This group is involved in hydrogen bonding in the Watson–Crick pair.

In the spectral range from 34600 to 36600 cm^{-1} investigated here we only found one prevalent A–T isomer. Further spectral regions will be investigated in future UV-UV and IR-UV measurements.

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