

IR-UV double resonance spectroscopy of guanine–H₂O clusters

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We present the IR-UV double resonance spectrum of guanine monohydrate in the region 3100 cm⁻¹ to 3800 cm⁻¹ along with the energies and frequencies of these structures calculated at the non-empirical correlated *ab initio* RI-MP2/cc-pVDZ level. We assign the structures of guanine–water clusters by comparing the experimental spectra with the *ab initio* calculations and with the IR spectra of the bare guanine monomer. We find two clusters with guanine in the enol-amino tautomeric form and one structure with guanine in the keto-amino form.

1. Introduction

In bulk solution, and in larger molecules of DNA and RNA, many forces influence structure. By isolating the nucleobases in the gas phase, it is possible to study microscopic effects independently and isolated from bulk effects, which normally mask the intrinsic molecular properties. In order for Watson–Crick pairing to occur guanine must exist in its keto-amino form, which is the main form found in solution.^{1,2} Experiments performed in the gas phase show the guanine monomer exists in both keto-amino and enol-amino forms.^{3,4} A number of theoretical studies have been aimed at describing the effects of water molecules on the purine nucleobases.^{5–9} Experimental studies that sequentially add water molecules to guanine may give insight into how the photophysics of this base is affected by its environment. Because of the relative difficulty of bringing the nucleobases into the gas phase and clustering them with water, few experimental studies have been performed which examine the effects of microscopic water on the structure of the bases.^{10–12} Kim *et al.* concluded that electronically excited clusters of adenine monomer with water dissociate within 200 fs as a result of vibronic coupling and a repulsive state of *n*– π^* character.^{13,14} He *et al.* recently examined the effects of water molecules on the S1 lifetimes of the pyrimidine bases and concluded that the presence of water molecules could increase the rate of internal conversion to the ground state through an intermediate dark state.¹⁵ Chin *et al.* observed just one structure, an enol-amino tautomer, for 9-methylguanine monohydrate under jet-cooled conditions, although calculations predict a keto structure to be very close in energy, just 0.45 kcal mol⁻¹ higher than the enol tautomer.¹¹ We are interested in examining the effects of step-wise hydration on the relative abundances of the guanine tautomers. In this study, we observe the effects of a single water molecule on the nucleobase guanine. We assign the structures of clusters of guanine and one water molecule (G + W) based on IR-UV double resonance experiments, *ab initio* calculations, and comparison to the jet-cooled IR spectra of guanine.

2. Experimental

All measurements were performed with an apparatus described in detail elsewhere.¹⁶ In brief, a thin layer of sample and ice are

laser desorbed from a graphite bar in front of a pulsed nozzle. The graphite sample bar is kept at liquid nitrogen temperatures by flowing N₂ through a copper tube in contact with the sample bar. The desorption laser, a Nd:YAG operating at 1064 nm, is attenuated to 1 mJ cm⁻² and focused within 2 mm in front of the nozzle opening. We use a pulsed valve with a nozzle diameter of 1 mm at a backing pressure of 5 atm of argon drive gas. The neutral molecules are ionized with a frequency-doubled dye laser and detected in a reflectron time-of-flight mass spectrometer. We obtain resonant two-photon ionization (R2PI) spectra by monitoring mass selected peaks while tuning the two photon, one color ionization wavelength. We measure UV-UV double resonance spectra with two laser pulses separated in time by 200 ns. Ionization laser intensities are approximately 3 mJ pulse⁻¹ and are attenuated to avoid saturation. The first pulse serves as a “burn” pulse, which removes the ground state population and causes depletion in the ion signal of the second “probe” pulse, provided both lasers are tuned to a resonance of the same isomer. IR-UV double resonance spectra are obtained in an analogous way with the burn laser operating in the near-IR region. IR frequencies ranging from 3100 cm⁻¹ to 3800 cm⁻¹ are produced in an OPO setup (LaserVision) pumped by a Nd:YAG laser operating at its fundamental frequency. Typical IR intensities in the burn region are 12 mJ pulse⁻¹.

3. Theoretical calculations

Four guanine tautomers (g17, g19, g9o1, g9o2; *cf.* Fig. 1A) are almost isoenergetic with relative stabilities of –0.50, 0.00, +0.29 and +0.09 kcal mol⁻¹.⁶ Structures of these tautomers as well as of the most stable monohydrates of these tautomers found by Hanus *et al.*⁶ were fully reoptimized at the RI-MP2/cc-pVDZ level.

Stabilization energies of all monohydrated structures depicted in Fig. 1B were determined as the sum of the complete basis set limit of RI-MP2 energies and a ($\Delta E_{\text{CCSD(T)}} - \Delta E_{\text{MP2}}$) correction term evaluated with the cc-pVDZ(0.25,0.15) basis set. The complete basis set limit of MP2 energies was determined by two-point Helgaker's¹⁷ extrapolation using the aug-cc-pVXZ basis sets for X = D and T, similarly as it is described in work by Jurečka and Hobza.¹⁸

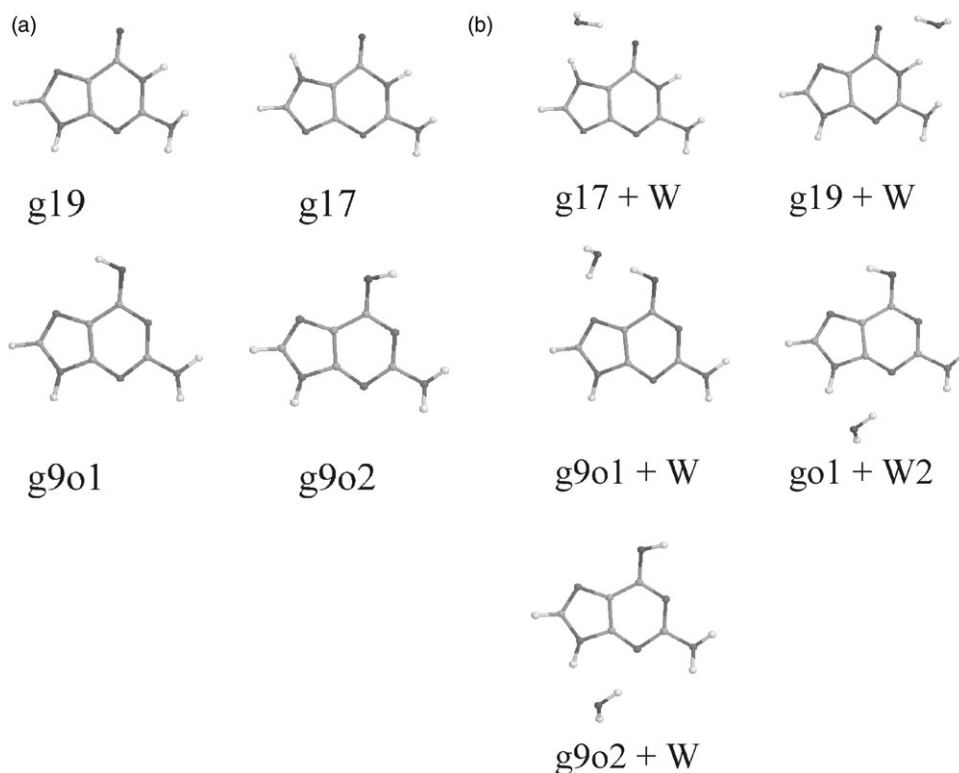


Fig. 1 (A) Four most stable guanine tautomers; (B) optimized structures of monohydrated guanine tautomers.

Harmonic vibration frequencies were evaluated numerically at the RI-MP2/cc-pVDZ level. They were scaled by a universal factor 0.956 to fit the experimental values of N–H stretch modes of isolated guanine.³ All calculations were carried out with the Turbomole 5.6 program.¹⁹ The visualizations of the theoretical IR spectra were generated by the Molden program.²⁰

4. Results

4.1. Theoretical calculations

Table 1 shows energy and enthalpy characteristics of monohydrated keto- and enol-guanine tautomers. The calculated scaled vibration frequencies of isolated bases and monohydrated bases appear in Tables 2 and 3, respectively. Fig. 1A shows the four lowest-energy tautomers of isolated guanine according to theoretical calculation and Fig. 1B depicts structures of the most stable guanine monohydrates.

Table 1 Thermodynamics characteristics (in kcal mol⁻¹) of monohydrated guanine complexes

	Δ RI-MP2 CBS ^a	Δ CCSDT ^b	Δ Ecorr ^c	Δ ZPVE ^d	Δ H ₀ ⁰ e
g17 + W	-13.64	0.64	-12.99	2.57	-10.43
g19 + W	-11.96	0.38	-11.58	2.47	-9.11
g9o1 + W	-10.81	0.41	-10.40	2.45	-7.95
g9o1 + W2	-11.08	0.59	-10.49	2.41	-8.08
g9o2 + W	-10.79	0.60	-10.20	2.33	-7.86

^a An interaction energy of the complex extrapolated to the complete basis set. ^b A correction for higher order correlation effects. Obtained as a difference between CCSD(T) and MP2 interaction energies. ^c A corrected interaction energy (sum of the second and third column). ^d Zero point vibration energy. ^e Enthalpy at 0 K (sum of the fourth and fifth column).

4.2. Mass spectrum

We present the mass spectrum of guanine in clusters of up to six water molecules in Fig. 2A. The mass spectrum of guanine dimers in clusters of up to six water molecules is shown for comparison to the guanine monomer in Fig. 2B. Relative abundances depend on the ionization wavelength to some degree but the following qualitative features persist for all wavelengths, covered in these experiments. The abundance ratio of the first cluster of guanine with water (G + 1W) to bare guanine is strikingly different from that of its first cluster of the dimer with water (GG + 1W) to the dimer parent. It is evident that the relative amount of G + W we observe is less than that of GG + 1W even though the intensity of the bare GG signal is approximately one half the intensity of the bare guanine monomer. This is intriguing given that Kim *et al.* observed similar behavior for the nucleobase adenine.¹⁴ They attribute this behavior to a rapid fragmentation of monomer clusters in the excited state caused by vibronic coupling of the π - π^* state to a dissociative n - π^* state. Our experiments on guanine differ in that we do see a small signal for monomer clusters with nanosecond excitation. It is suspected that the π - π^* state of guanine lies lower in energy than the π - π^* state of adenine.²¹ The fact that we do observe a signal for guanine monomer with water may be explained by a slightly weaker coupling between the π - π^* and repulsive n - π^* states in these

Table 2 Scaled (by factor 0.956) harmonic frequencies (in cm⁻¹)/relative intensities (in km mol⁻¹) of isolated tautomers of guanine

	NH2 a ^a	NH2 s ^b	N1-H1	N7-H7	N9-H9	GOH ^c
g17	3504/21	3396/26	3453/58	3505/130	—	—
g19	3518/37	3407/35	3451/56	—	3506/87	—
g9o1	3557/36	3434/59	—	—	3507/102	3602/95
g9o2	3573/32	3457/47	—	—	3512/103	3602/104

^a Asymmetric stretch of amino group. ^b Symmetric stretch of amino group. ^c Stretch of OH of guanine.

Table 3 Scaled (by factor 0.956) harmonic frequencies (in cm^{-1})/intensities (in km mol^{-1}) of monohydrated tautomers of guanine

NH2 a ^a	NH2 s ^b	N1-H1	N7-H7	N9-H9	GOH ^c	WOH free ^d	WOH HB ^e
3500/31	3396/57	3453/59	3167/679	—	—	3724/89	3445/506
3529/41	3415/41	3191/479	—	3506/122	—	3724/84	3464/379
3571/38	3440/43	—	—	3509/102	3105/913	3716/87	3384/909
3562/17	3440/53	—	—	3325/335	3602/98	3726/84	3495/416
3560/19	3439/48	—	—	3327/337	3596/95	3727/85	3498/423

^a Asymmetric stretch of amino group. ^b Symmetric stretch of amino group. ^c Stretch of OH group of guanine. ^d Stretch of OH group of water which is not involved in H-bond. ^e Stretch of OH group of water which is involved in H-bond.

clusters, as compared to adenine. These observations suggest that complexation of guanine with water decreases its excited state lifetime and dimerization appears to counteract this mechanism.

4.3. REMPI

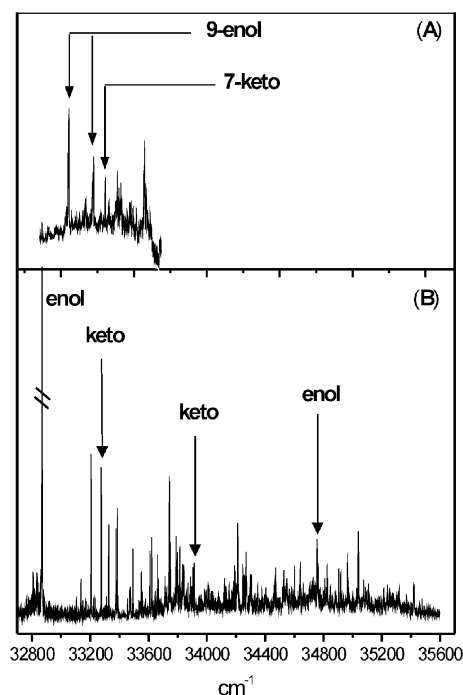
In Fig. 3 we present the R2PI spectra of guanine (G) and guanine monohydrate (G + W).²² IR-UV double resonance and substitution experiments have identified four gas phase tautomers of guanine.^{3,4,10,23} These include two keto and two enol tautomers. The $\pi-\pi^*$ origin transitions of these tautomers are labeled in Fig. 3B. The R2PI spectrum of the cluster of G + W appears in Fig. 3A and its origin transitions are labeled. Evidence for the identification of these peaks as arising from enol or keto tautomers is given in section 4.5. There is a very small peak at $32\,869\text{ cm}^{-1}$ that we did not probe with double resonance spectroscopy and that could be due to an $n-\pi^*$ transition, analogous to that observed in the monomer,¹⁰ or possibly to a hot band.

4.4. UV-UV double resonance spectroscopy

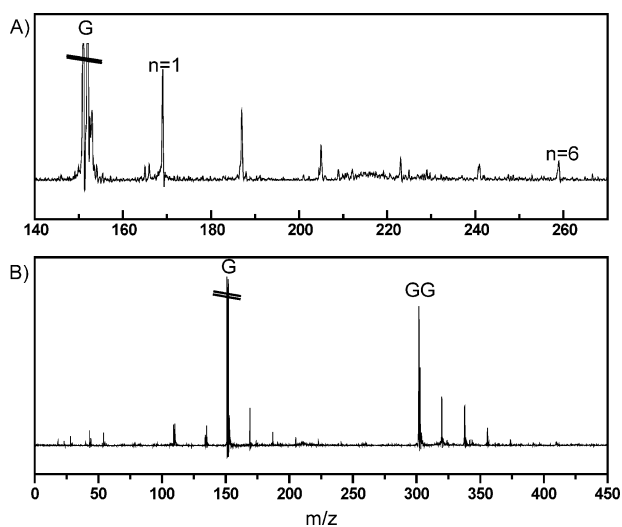
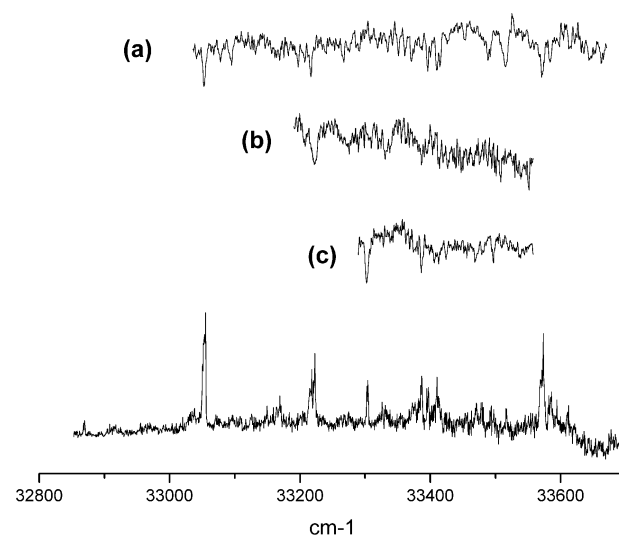
UV-UV double resonance experiments previously identified three different isomers for guanine monohydrate.¹⁰ For completeness, we present here the UV-UV double resonance spectra for G + W from $33\,000\text{ cm}^{-1}$ to $33\,650\text{ cm}^{-1}$, obtained in our own lab, in Fig. 4. The three isomer spectra are labeled as (a), (b), and (c), and in the following sections we present the IR spectra for these clusters and make structural assignments.

4.5. IR-UV double resonance spectroscopy

Fig. 5 shows the IR-UV double resonance spectra of each of the three isomers of G + W together with the IR spectra of

**Fig. 3** R2PI spectra of (A) guanine + 1 water, (B) guanine monomer.

three guanine monomers, in the 9-enol, the 9-keto and the 7-keto form for comparison. The IR spectra of the three guanine hydrates were obtained by tuning the ionization or “probe” laser to $33\,049\text{ cm}^{-1}$, $33\,218\text{ cm}^{-1}$, and $33\,301\text{ cm}^{-1}$, corresponding to origins in the UV-UV traces (a), (b), and (c) in Fig. 4, respectively. The IR spectrum in trace (b) contains peaks

**Fig. 2** Mass spectra of (A) guanine + $n\text{H}_2\text{O}$ with up to $n = 6$ water molecules (B) guanine clusters with water (G) and guanine dimer clusters with water (GG). The mass signal of guanine has been cut in order to scale up the other mass peaks.**Fig. 4** UV-UV double resonance spectra of guanine monohydrate (G + W).

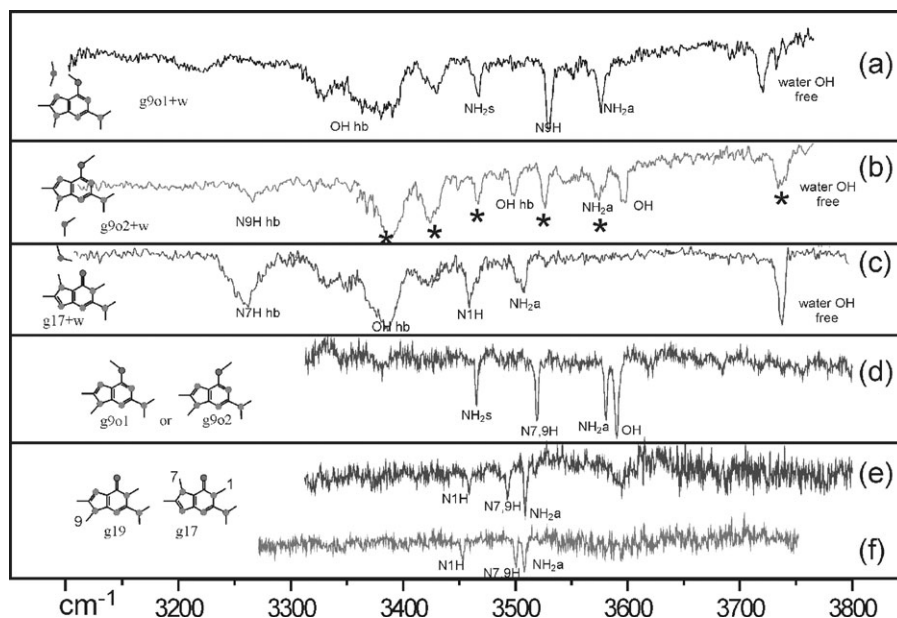


Fig. 5 IR-UV double resonance spectra of guanine monohydrate (a)–(c) and guanine monomer (d)–(f). The structure diagrams are merely schematic, and refer to tentative assignments discussed in more detail in the text. Asterisks indicate peaks in trace (b) that also appear in trace (a), possibly as a result of partial overlap in the double resonance UV probe.

that belong to the structure of trace (a) because of the close proximity in the UV spectra of the origin of (b) to a vibronic transition of (a). This makes it very difficult to probe the former without also probing the latter. Since it is possible to measure a clean spectrum for (a) by tuning the UV probe to its origin transition at $33\,050\text{ cm}^{-1}$, we can determine the peaks in (b) that might arise from isomer (a) and we can exclude them from the analysis.

Inspection of the IR spectra of G + W complexes reveals that all three structures exhibit strong transitions within 10 cm^{-1} of 3740 cm^{-1} . This transition corresponds to the free OH stretch mode of the water molecule and therefore we can rule out any clusters in which both OH of the water are bound. IR spectrum (a) very much resembles the spectrum of the enol monomer of guanine (d). Based on the IR frequencies alone the monomer could be either the 7H enol or 9H enol tautomer, since the N7H and N9H frequencies are virtually identical. Further considerations on this assignment will be discussed below. The NH_2 asymmetric, N9H, and NH_2 symmetric modes appear at almost the same frequencies as in the monomer, which suggests guanine is in its enol form. The free OH stretch of the enol form of guanine at 3590 cm^{-1} does not appear since it participates in a hydrogen bond to the oxygen of water. This can shift the OH stretch frequency by $150\text{--}300\text{ cm}^{-1}$, which places this stretch in the same region as the bound OH of water, or even slightly more to the red as calculation predicts. We assign the broad peaks between 3300 cm^{-1} and 3450 cm^{-1} to the bound water OH modes. Experiments performed in our lab on the guanine dimer + $2\text{H}_2\text{O}$ are consistent with this peak assignment of the bound OH of water.¹² The peak at 3216 cm^{-1} may be assigned to the bound OH of the enol group. Based on these considerations, we assign structure (a) as $\text{g9o1} + \text{W}$, the 9-enol guanine with a water molecule bound to the OH of the enol group and N7. The second spectrum in Fig. 5 also shows strong NH_2 asymmetric and symmetric bands, characteristic of guanine in the enol form, as well as the free enol OH stretch mode at 3597 cm^{-1} . As discussed above, there is overlap with the spectrum of structure (a) in the IR-UV double resonance data and so we exclude from the analysis the peaks, marked with an asterisk, that are shared with trace (a). These peaks include the broad peaks corresponding to bound OH groups centered at 3426 cm^{-1} and 3382 cm^{-1} , and the N9H unbound mode appearing at 3525 cm^{-1} .

The bound water OH in this structure appears at 3500 cm^{-1} , shifted approximately 150 cm^{-1} compared to a free enol stretch. We believe that the hydrogen bond formed between the N3 guanine and OH of water is not that strong, which

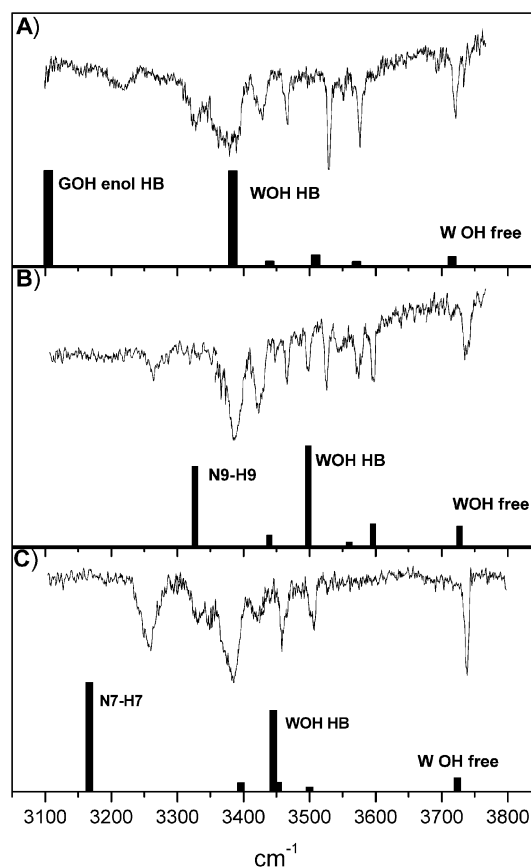


Fig. 6 IR-UV double resonance spectra of three guanine–water cluster isomers compared with theoretical computations for the respective structures discussed in the text. GOH and WOH refer to guanine and water OH stretches, respectively, HB indicates a hydrogen bond mode as opposed to a free mode. Asterisks indicate peaks in trace (b) that also appear in trace (a), possibly as a result of partial overlap in the double resonance UV probe.

explains the moderate shifting and broadening of this mode compared to the bound water OH modes in the other conformers. Theoretical calculations on the frequency show that this mode matches the assignment which can be clearly seen in Fig. 6. The N9H peak is red-shifted by almost 300 cm^{-1} to 3364 cm^{-1} , consistent with water binding at this site. We thus assign IR spectrum (b) as $g901a + W$ or $g902 + W$, two OH rotamers of the 9-enol guanine with a water molecule bound to N3 and N9H. We cannot distinguish these two structures based on the theoretical calculations of their stability (*cf.* Table 1) and vibrational frequencies (*cf.* Table 3).

The third conformer of guanine monohydrate, (c), appears similar to the guanine monomer in the keto form, (e) or (f), with the N7H/N9H stretch missing and a single free OH stretch. This suggests a structure with one water OH bonding with the C=O and the water O bound to an NH, which then has to be the N7H. The N1H mode appears at the same frequency in (c) as it does in the keto forms of the monomer. The NH_2 asymmetric mode also remains unchanged from the monomer keto form, which is expected if guanine is in the keto form and also eliminates this group as a possible hydrogen bond participant. The broad peak appearing at 3386 cm^{-1} is consistent with the bound OH stretch of water. We thus assign structure (c) to $g17 + W$.

Fig. 6 compares the experimental spectra with the calculated frequencies and intensities for the three structures discussed above. The experimental relative intensities are not accurate because of saturation in the double resonance spectra but they do correspond qualitatively with the calculations. The comparison of the experimental IR frequencies to theoretical calculation shows good agreement.

5. Discussion

As described above, we have performed tentative structural assignments of the three cluster structures we found for $G + W$, based on comparisons of their double resonant IR spectra with those of the monomers. We have found excellent agreement of these spectra with the calculated spectra for the three tentatively assigned structures. However, the differentiation between the N7H vs. N9H tautomer is not straightforward, since frequencies of those NH stretches are very similar.³ This is particularly important, because our choice of structures does not include the N9H-keto tautomer, which is biologically most relevant. The same tautomer is missing in all observed spectra of 9-substituted guanines, including guanosines, so far.^{4,11,24,25} As shown in Table 1, according to the calculations, the three N9H enol and the two N7H/N9H keto clusters with water constitute the lowest energy structures. Structure (c) is the only keto cluster we have found so far, and its assignment is fairly unambiguous. The free N7H or N9H mode is missing, while there is only one free water OH. The only low energy keto cluster structure that is consistent with these conditions is $g17 + W$, since it allows the water to hydrogen bond simultaneously with the C=O and the N7H. We cannot propose any reasonable N9H keto + W structure in which the water bonds with N9H. In the guanine monomer the origins of the two keto tautomers $g17$ and $g19$ are split by 638 cm^{-1} so it is possible that a N9H-ketoguanine + water structure exists with its UV origin outside the range observed by us so far.

For the enol structures the assignment is not entirely unambiguous because it is possible to conceive of an N7H structure that could also be consistent with the data. According to the calculations of Hanus *et al.*⁶ the lowest energy N7H enol + W, which would have a very similar IR spectrum, would be 4.5 kcal mol^{-1} higher in energy than in the $g901$ case. A similar dilemma exists for the monomer. Spectrum (d) in Fig. 5 cannot be assigned as N7H or N9H on the basis of the IR frequencies alone. Nir *et al.*³ chose the N9H assignment, solely based on the fact that constitutes the energetically lower form.

However, relative abundances in these experiments may be poorly correlated with calculated stabilities. Mons *et al.*⁴ compared origin positions with those of 7- and 9-methyl substituted guanines and concluded that the red-most origins of both the enol and keto tautomers are of the N7H form and the blue-most origins of the N9H form. Following the same logic, we note in Fig. 3 that the origins of the enol hydrates are closest to the origin of the red-most enol monomer. In other words, if the red-most origin of the monomer corresponds to the N7H enol tautomer, in spite of its having a significantly higher energy than the N9H enol tautomer, then the same could be true for the enol hydrates. As noted, the assignment of spectrum (c) as the N7H keto hydrate is consistent with the monomer assignment of Mons *et al.*⁴ We do observe that if the hydrate enol structures are correlated with the red-most origin of the monomer structures (Fig. 3) then the clustering with one water molecule appears to cause a blue-shift of the electronic origin. This would also be true if this is the keto hydrate although the effect in that case would be less pronounced. This is somewhat unusual compared, for example, with water clusters of molecules such as phenol, which exhibit a red-shift in the $\pi-\pi^*$ transition.²⁶⁻²⁹ It is possible that the second hydrogen bond, in which the water acts as a proton donor rather than a proton acceptor, plays a role in the present case. We note that Chin *et al.* observed a similar blue-shift for the cluster of 9-methylguanine with water.¹¹

6. Conclusion

We observe three different conformers of guanine monohydrate in the gas phase. We make complete structural assignments of these conformers based on IR-UV double resonance spectroscopy and *ab initio* calculations. The three structures include the 7-keto hydrate and two of the enol hydrates, which differ in the position of the water molecule. We do not find a guanine 9-keto hydrate, which is the second most stable structure predicted by theoretical calculation. It is possible that the electronic origin of this cluster is outside the region investigated in this experiment, particularly further to the red. Furthermore, differences in excited state lifetimes may play a role in the selective observation of specific structures. We also note a very small ratio of cluster signal to monomer signal, which may arise from a decrease in the lifetime of the S1 state. These experiments are part of a larger study in which we are examining the influence of microscopic effects of water on the structure of nucleobases and their clusters. Experiments that add more water molecules, up to the first hydration shell, will be helpful in determining the effects of water on the tautomers of guanine.

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