IR-UV Double Resonance Spectroscopy of the Hydrated Clusters of Guanosine and 9-Methylguanine: Evidence for Hydration Structures Involving the Sugar Group

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Infrared spectra of mono- and dihydrated clusters of guanosine (Gs) formed by laser desorption combined with supersonic jet cooling have been measured by the technique of IR-UV double resonance spectroscopy. The results are compared with those of 9-methylguanine (9MG), in which the sugar group of Gs is replaced with a methyl group, to elucidate the importance of the sugar group in the hydration structures. It is shown that the UV spectrum observed for the monohydrated cluster of Gs is composed of multiple structural isomers of larger stabilities. One of the monohydrates is identified to possess a hydration structure involving the 5′-OH group of the sugar and the amino group of the guanine moiety. The IR spectrum of the dihydrated clusters reveals that the 2′-OH group is significantly influenced by the addition of the second water, which suggests the possibility of specific dihydrate structures for Gs.

1. Introduction

The presence of water is essential to the conformational stabilization and interaction of nucleic acids (NA).[1] The X-ray crystallography of DNA has revealed that a shell of water molecules is tightly bound to the NA components and reveals different properties from those of the bulk water.[2−5] It is also believed that these water molecules play an important role in the recognition process of specific DNA sequences by proteins. In contrast to DNA, the hydration behavior of RNA has not been investigated extensively, although the conformational rigidity of RNA is often explained by the hydration to the 2′-hydroxy group of ribose.[6]

Hydrated clusters of the NA components and other biological molecules formed in the gas phase are expected to provide important information of the hydration structure by offering unique environments for studying microscopic hydrogen-bonding interactions.[7,8] Monohydrated clusters of guanine and 9MG were formed by laser desorption, and their hydrogen-bonding structures were identified by IR-UV double resonance spectroscopy.[9,10] In our previous study,[11] mono- and dihydrated clusters of guanosine (Gs) and 2′-deoxyguanosine were generated by the combination of laser desorption and supersonic jet-cooling techniques, and their UV spectra were recorded by resonant two-photon ionization (R2PI) spectroscopy. Qualitative structural assignments of these hydrates were given on the basis of the low-energy isomeric forms obtained from theoretical calculations.

In this work, we have employed the technique of IR-UV double resonance spectroscopy to obtain infrared spectra of the mono- and dihydrated clusters appearing in the UV spectra. The results are found to be consistent with the previous structural assignments for the monohydrated clusters derived from the UV spectral signature and theoretical calculations.

2. Experimental and Computational Methods

The pulsed laser desorption method employed in this study has been described elsewhere.[11,12] Laser desorption was accomplished by irradiating a sample pellet placed inside the source by the second harmonics of a YAG laser. The pellet was prepared by mixing sample powder with graphite (5%) and by pressing with a hydraulic press. Hydrated clusters of Gs and 9MG were formed by passing Ar carrier gas (5 atm) through a water reservoir. Mass-selected UV spectra were measured by the resonant two-photon ionization (R2PI) method as described previously.[12] Infrared spectra of hydrated clusters were recorded in the frequency range 3000−3700 cm^{-1} by the scheme of IR-UV double resonance spectroscopy. The IR beam (1−3 mJ/pulse) was provided with an OPO laser (LaserVision) pumped by a YAG laser.

The DFT/B3LYP method with a 6−31++G** basis set was used for investigating stable geometries of the mono- and dihydrated clusters. Thereafter, single-point calculations at the MP2/6−31++G** level were carried out for the lower energy structures to improve the accuracy of the energetic ordering of the isomeric forms. IR spectra were calculated at the B3LYP/6−311++G** level for the lower energy structures.

3. Summary of R2PI Spectra

One-color R2PI spectra obtained by monitoring the mass channels of Gs^+ and those of its monohydrate ion GsW_1^+ and dihydrate ion GsW_2^+ are displayed in Figure 1. The bottom spectrum measured in the Gs^+ mass channel was obtained with water entrained in the Ar carrier gas. A series of sharp bands located around 34 500 cm^{-1} in the bottom spectrum match the spectrum of Gs monomer reported by Nir et al.[13,14] Its origin is marked with an asterisk (*). The low-frequency modes were assigned to the mutual motions of the sugar group and guanine moiety. Results of UV−UV and UV-IR double resonance measurements indicated that this R2PI spectrum originates from an enol form of guanine, and the 5′-OH group of the sugar ring and the N3 atom of the guanine are hydrogen-bonded together.

In our previous work,[11] the R2PI spectrum of GsW_1 shown in Figure 1b was assigned as a composite of several different isomeric forms. A series of narrow bands appearing in the energy region below <34 200 cm^{-1} correspond to the spectral features of one isomer. The red-most peak is shifted by −653

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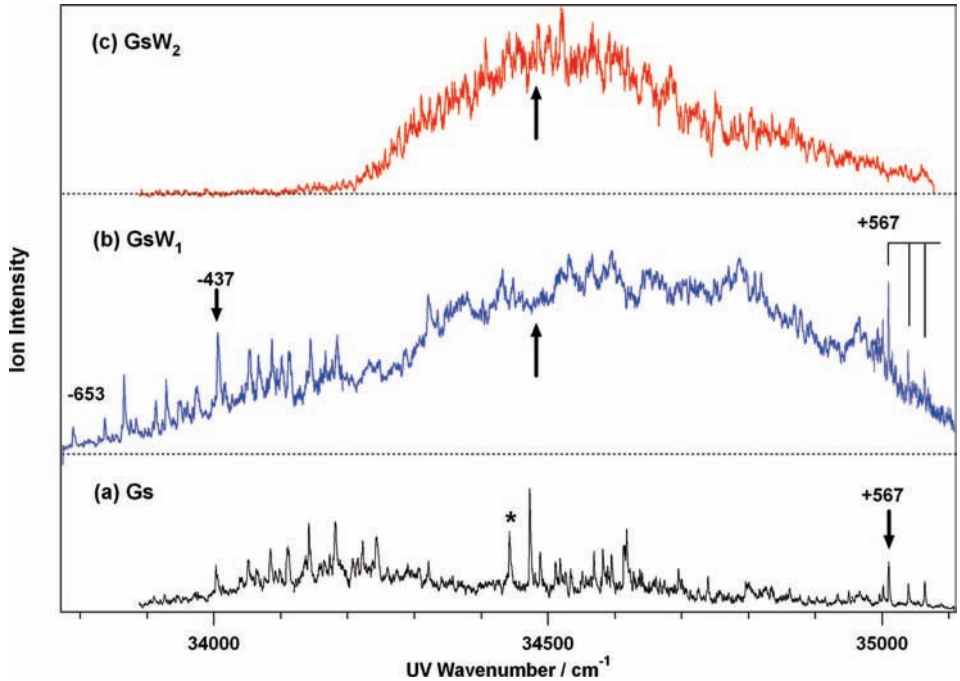

Figure 1. R2PI spectra obtained at the mass channels of (a) Gs^+, (b) GsW_1^+, and (c) GsW_2^+. The asterisk in part a indicates the origin band of the Gs monomer. The spectral shifts (cm^{-1}) for the pronounced peaks of the monohydrates from this origin band are indicated in part b. The arrows mark the UV transitions to record IR-UV double resonance spectra.

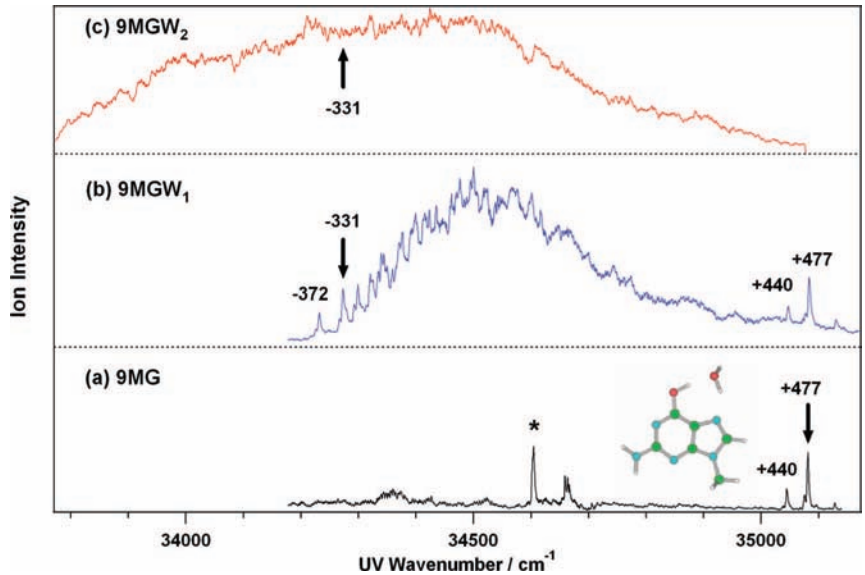

Figure 2. R2PI spectra obtained at the mass channels of (a) $9MG^+$, (b) $9MGW_1^+$, and (c) $9MGW_2^+$. The asterisk shown in part a indicates the origin band of the 9MG monomer. The arrows mark the UV transitions to record IR-UV double resonance spectra. The monohydrate structure (aW67) identified in ref 15 is shown as an inset.

cm^{-1} from the monomer origin and is followed by several peaks at irregular frequency intervals. At a higher energy region of 35 000 cm^{-1}, a couple of sharp features are noticeable. The most prominent peak is blue-shifted by 567 cm^{-1} from the band origin of Gs monomer. They are associated with a second monohydrate structure. Furthermore, it is likely that the broad bands appearing in the region between 34 200 and 35 000 cm^{-1} are due to other monohydrates of lower energies.

The R2PI spectrum measured in the Gs^+ mass channel (Figure 1a) exhibits sharp bands (e.g., peaks at −437 and +567 cm^{-1}). Since these spectral features match those of the GsW_1 spectrum, they are associated with those of larger clusters. This observation is explained by the evaporation of water molecule upon ionization. In contrast, the broad bands observed in the 34 500 cm^{-1} region of the GsW_1 spectrum are not significant in the spectrum measured at the Gs^+ ion channel. The spectrum measured at the GsW_2^+ mass channel in Figure 1c appears to be similar to the broad spectral region of GsW_1. It consists only of broad features with no significant intensity in the energy region below 34 200 cm^{-1}. This similarity suggests the possibility of fragmentation of the dihydrates into this GsW_1^+ ion.

Figure 2 shows the R2PI spectra obtained at the mass channels of $9MGW_1^+$ and $9MGW_2^+$ ions. The bottom spectrum is obtained by measuring $9MG^+$ monomer ions in the presence of water vapor. Chin et al.[15] assigned the prominent peak marked with an asterisk in Figure 2a to the enol form with its hydroxy group oriented toward the N7 atom (i.e., antienol form). The spectrum of $9MGW_1$ in Figure 2b reveals two prominent peaks

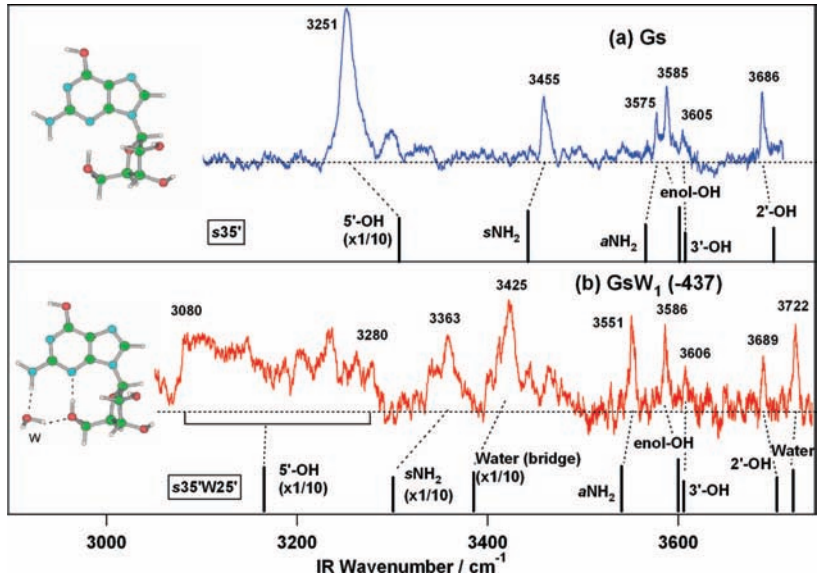

Figure 3. IR-UV double resonance spectra of (a) Gs and (b) GsW_1. The IR spectrum of Gs was obtained in the absence of water vapor while that of GsW_1 was recorded by probing through the -437 cm^{-1} band in Figure 1b, respectively. Calculated IR spectra (scaled by 0.957) are shown for the syn-enol form of Gs and the corresponding monohydrate (s35′W25′, see Figure 4g).

TABLE 1: IR Frequencies Observed for Gs and GsW_1[a]

Gs	GsW_1			assignment[e]
	-437 cm^{-1} [b]	broad band[c]	$+567$ cm^{-1} [d]	
	3722	3711, 3722	3712	OH (water)
3686	3689	3689	3689	2′-OH
3605	3606	3605	3607	3′-OH
3585	3586			OH (enol)
3575	3551	3545[f]	3578	NH_2 (anti)
3455	3363[f]	3430[f]	3458	NH_2 (sym)
	3425[f]	3269[f]	3275[f]	OH (water bridge)
3251[f]	3080−3280[f]		3226[f]	5′-OH
			3095−3125[f]	OH (enol bridge)

[a] All frequencies are in cm^{-1}. [b] Transitions probed through -437 cm^{-1} peak in Figure 1b. [c] Transitions probed through the broad band in Figure 1b. [d] Transitions probed through $+567$ cm^{-1} peak in Figure 1a. [e] Assignment for GsW_1 is tentative because of the possible existence of multiple isomers. [f] Broad transition.

blue-shifted by 440 and 477 cm^{-1} from the monomer origin. The monohydrate was identified as the same antienol form with the water molecule bridging the O6H proton and the N7 site, which is labeled as aW67 and is shown in Figure 2a. This corresponds to the most stable monohydrate structure of 9MG. However, spectral features due to the keto forms of 9MG and its monohydrate are apparently absent from the respective R2PI spectra. The failure to observe these species by nanosecond-laser-based R2PI was explained by the occurrence of specific ultrafast excited-state dynamics.[15]

In the spectrum of $9MGW_1$ in Figure 2b, many narrow bands are observed to the red of the monomer origin. The red-most peak at 34 232 cm^{-1} is red-shifted by 372 cm^{-1} from the monomer origin. These red-shifted peaks were not recognized by Chin et al.[15] They are accompanied by a broad background feature whose intensity increases dramatically with increasing excitation energy. On the basis of theoretical consideration, we suggested that these features are those of the monohydrate of the missing keto tautomer.[11] In this monohydrate, the water molecule links the oxygen atom of the C═O group and the N1H site to form a six-membered ring, which is labeled as kW16. This hydrate is nearly isoenergetic to the aW67 form and is more stable by 10 kJ/mol than that of the syn-enol form (sW16).

The spectrum obtained by measuring monomer ions of $9MG^+$ exhibits the sharp peaks in the higher energy region that match those of $9MGW_1^+$ in Figure 2b. This observation is similar to the case of Gs and is explained by the loss of the water molecule upon ionization. In contrast, the red-shifted features observed at the $9MGW_1^+$ mass channel are nearly absent from the spectrum of $9MG^+$. At first glance, it appears that the red-shifted spectrum is due to fragmentation of larger clusters upon ionization. Nevertheless, as described below, the IR-UV double resonance result shows that the $9MGW_1^+$ ion signal obtained following excitation into these spectral features is associated with the corresponding neutral species.

The R2PI spectrum detected at the $9MGW_2^+$ mass channel in Figure 2c is further red-shifted from that of the monohydrate and reveals only broad bands. It was suggested that this dihydrate corresponds to the lowest energy structure since all other dihydrated structures are less stable by >22 kJ/mol.[11] In this dihydrate, the guanine moiety is in the keto form, and two water molecules form a dimer and bridge the oxygen atom of the C═O group and the N1H site (labeled as kWW16). This water dimer motif is consistent with the theoretical results for 9H tautomer of guanine.[16]

4. Results and Discussion

In this section, microscopic hydration structures of Gs are discussed on the basis of the results of IR-UV double resonance spectroscopy and comparison with those of 9MG.

IR Spectra of GsW_1 Probed through Red-Shifted UV Band. Figure 3 shows the IR spectrum obtained by probing through the red-shifted peak at -437 cm^{-1} in Figure 1b as compared to that of the Gs monomer. The IR spectrum of the monohydrate exhibits a sharp transition at 3586 cm^{-1} that can be assigned to the free OH stretch of the enol tautomer of guanine (enol-OH). This transition is therefore nearly unchanged upon hydration. In addition, the two amino stretches of the monomer (3575 and 3455 cm^{-1}) are substantially shifted to lower frequencies in the monohydrate (3551 and 3363 cm^{-1}). The intense transitions of the monomer observed around 3250 cm^{-1} are due to 5′-OH stretch. Its strong red-shift with respect to 2′-OH and 3′-OH is consistent with the presence of the

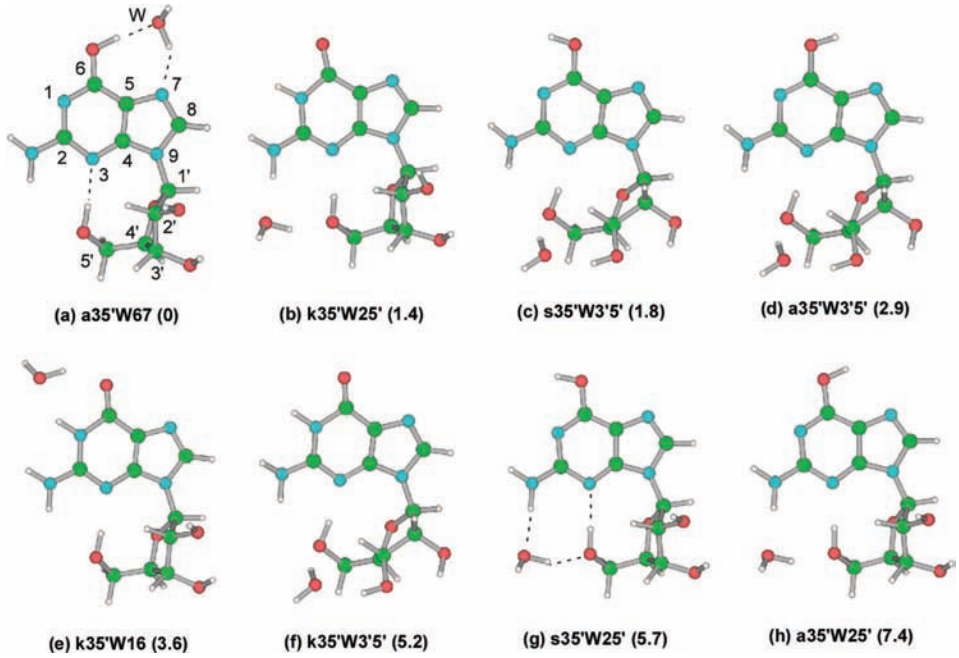

Figure 4. Low-energy monohydrate structures of Gs identified by the DFT/B3LYP calculation with a 6−311++G** basis set. The relative energies are indicated in kJ/mol. The letters "a", "s", and "k" indicate tautomers with the guanine moiety in the antienol, syn-enol, and keto forms, respectively. The label 35′ indicates the internal hydrogen bonding between the 5′-OH group of the sugar ring and the N3 atom of the guanine, which is retained in all hydrates. The last two numbers refer to the sites of the guanine moiety or the sugar group linked by the water (W).

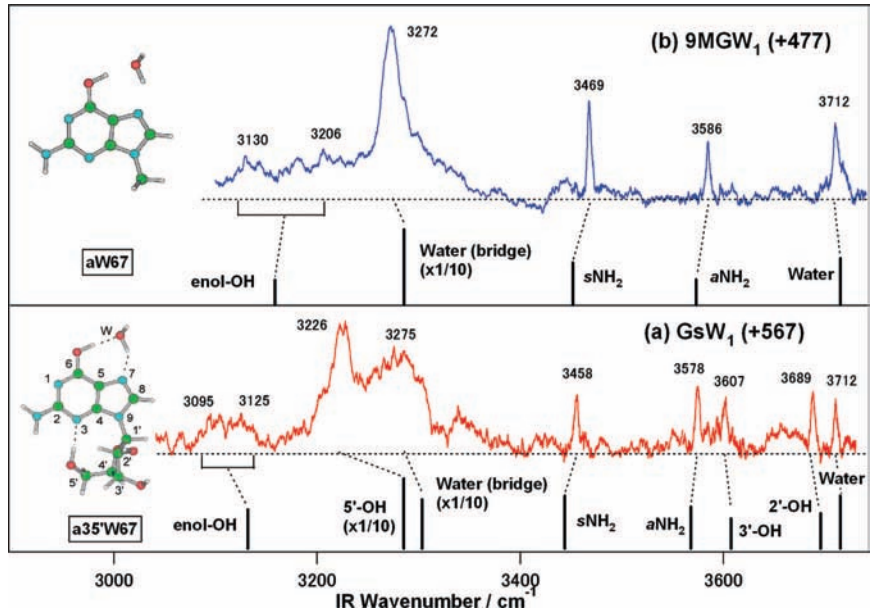

Figure 5. IR-UV double resonance spectra of (a) GsW_1 and (b) $9MGW_1$ obtained by probing through the UV bands of +567 cm^{-1} in Figure 1a and +477 cm^{-1} in Figure 2a, respectively. Calculated IR spectra (scaled by 0.957) are shown for the respective a35′W67 and aW67 structures.

internal hydrogen bonding with the N3 atom of the guanine. This transition is apparently absent from the monohydrate spectrum while those of other OH stretches of the sugar group are unaffected. These observations firmly establish the involvement of both amino and 5′-OH groups in hydrogen bonds with water. The frequencies of the observed IR transitions are listed in Table 1.

Figure 4 displays the low-energy monohydrate structures of Gs obtained by the DFT/B3LYP calculation with a 6−311++G** basis set. Among the monohydrates of the enol form, the cyclic structures in which water bridges N2H of the guanine moiety and 5′-OH of the sugar (labeled as W25′) are consistent with the red-shifts of the NH_2 stretches. Furthermore, the enol-OH stretch frequency appears to be lower than that of the 3′-OH stretch. The result of frequency calculation (see below) obtained at the B3LYP/6−311++G** level indicates that this ordering is reversed if the hydroxy group of guanine is oriented toward the N7 site (see Figure 7h). In addition, the intensity of the 3′-OH transition is calculated to be weaker than that of the enol-OH. Thus, this hydrate is assigned to be based on the syn-enol form of guanine s35′W25′ (Figure 4g), where the label 35′ designates the internal hydrogen bonding of the 5′-OH and the N3 site. This structure is calculated to be higher in energy by 5.7 kJ/mol than the most stable structure a35′W67. However, the corresponding antienol rotamer a35′W25′, which is slightly less stable, is not noticeable in the UV spectrum.

The IR spectrum calculated for the s35′W25′ monohydrate is displayed in Figure 3. The red-shifts of the two amino stretch

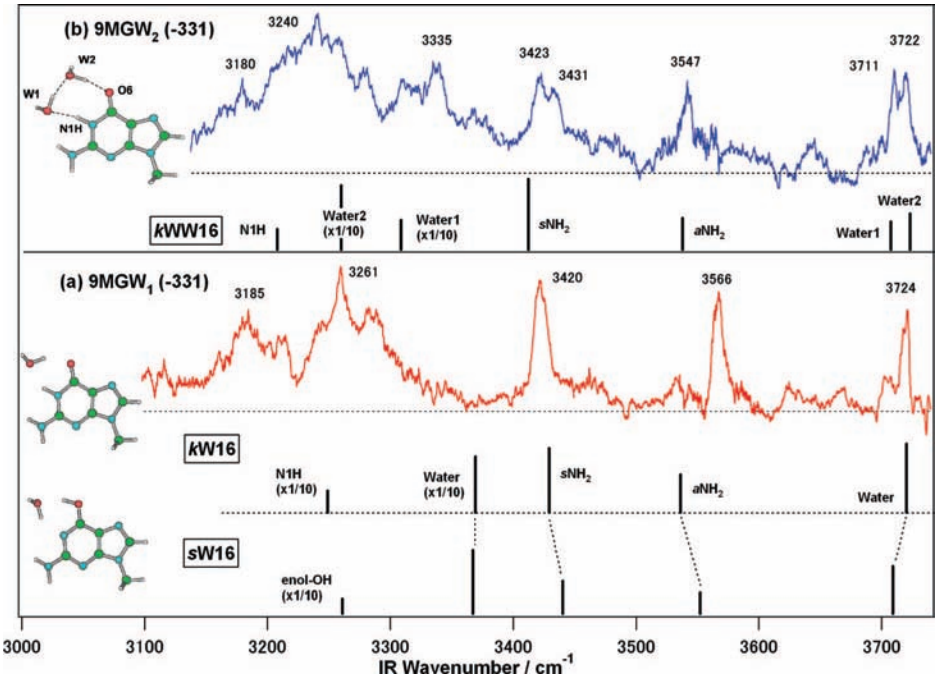

Figure 6. IR-UV double resonance spectra of (a) $9MGW_1$ and (b) $9MGW_2$ obtained by probing through the red-shifted UV bands in Figure 2b and 2c, respectively. Calculated IR spectra (scaled by 0.957) are shown for the kW16 and sW16 structures of $9MGW_1$ and for the kWW16 structure of $9MGW_2$.

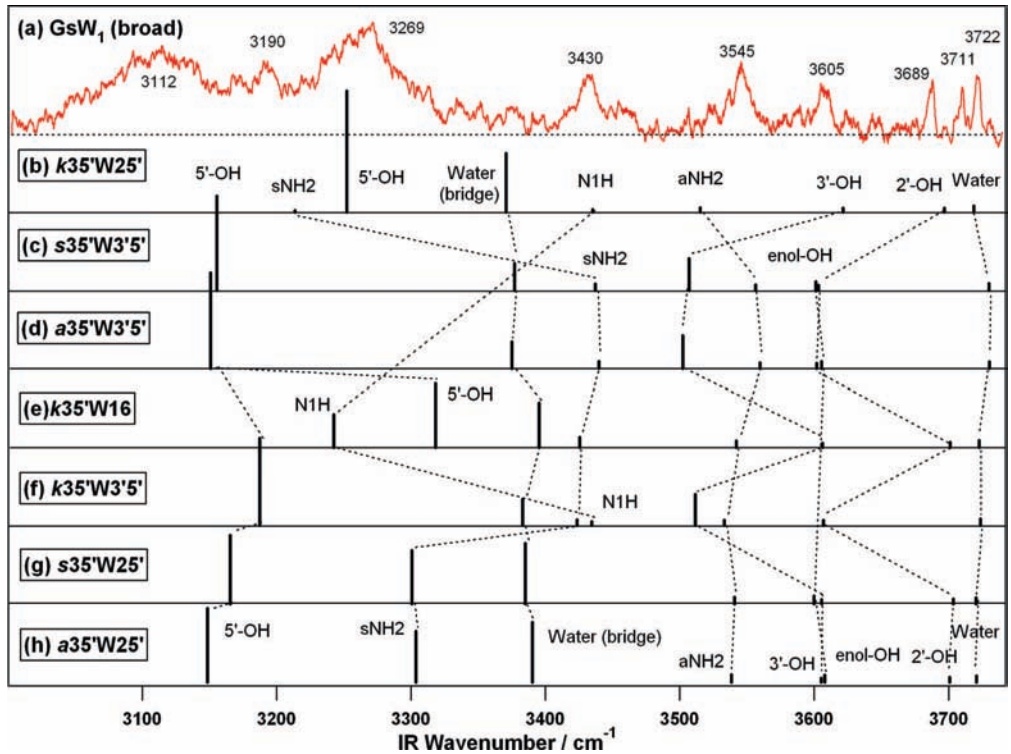

Figure 7. (a) IR-UV double resonance spectrum of GsW_1 obtained by probing through the broad UV band around 34 500 cm^{-1} in Figure 1b. (b−h) Calculated IR spectra (scaled by 0.957) for the low-energy structures b−h are displayed in Figure 4.

frequencies upon hydration on this group are nicely reproduced by the calculated IR spectra. The 5′-OH stretch frequency for this cyclic structure is calculated to be 3165 cm^{-1}, which agrees well with the appearance of broad bands in the range 3080−3280 cm^{-1}.

IR Spectra of GsW_1 and $9MGW_1$ Probed through Blue-Shifted UV Bands. Figure 5 compares the IR-UV double resonance spectra of the monohydrates of Gs and 9MG obtained by probing through the blue-shifted peaks at +567 cm^{-1} in Figure 1a and +477 cm^{-1} in Figure 2a, respectively. These peaks are attributed to the monomer fragments (Gs^+ and $9MG^+$) arising from dissociation of the respective monohydrates. We have also recorded IR spectra by probing through the corresponding peaks in the monohydrate spectra. However, these peaks are accompanied by broad background features as shown in Figures 1b and 2b thus complicating the spectral analysis.

The IR spectrum for $9MGW_1$ agrees with that of Chin et al., which was assigned to the most stable monohydrate of the

TABLE 2: IR Frequencies Observed for 9MG, $9MGW_1$, and $9MGW_2$[a]

9MG	$9MGW_1$		$9MGW_2$	assignment[d]
	-331 cm^{-1} [b]	$+477$ cm^{-1} [c]		
			3722	OH (water 2)
	3724	3712, 3724	3711	OH (water 1)
3595				OH (enol)
3587	3566	3586	3547	NH_2 (anti)
3470	3420	3469	3423	NH_2 (sym)
	3261[e]	3272[e]	3335[e]	OH (water 1 bridge)
			3240[e]	OH (water 2 bridge)
		3130−3206[e]		OH (enol bridge)
	3185[e]		3180[e]	N1H

[a] All frequencies are in cm^{-1}. [b] Transitions probed through -331 cm^{-1} peak in Figure 2b. [c] Transitions probed through $+477$ cm^{-1} peak in Figure 2a. [d] Assignments for $9MGW_1(-331$ cm$^{-1})$ and $9MGW_2$ are based on the assumption of the keto forms. [e] Broad transition.

antienol form aW67 (shown as an inset in part b). It can be seen that the IR spectrum of GsW_1 corresponds well to that of $9MGW_1$. This suggests a similarity in the hydration structures of the two monohydrates. Also, additional peaks are present in the spectrum of GsW_1. They are assigned to the OH stretching vibrations of the sugar group, which are essentially unaffected upon hydration. The fact that the transition due to 5′-OH is strongly shifted and broadened with respect to 2′-OH and 3′-OH can be explained by the persistence of the strong internal hydrogen bonding with the N3 site. The symmetric and antisymmetric stretching vibrations of NH_2 are also unchanged indicating that this group is not involved in the hydration. These observations firmly support that this monohydrate of Gs corresponds to the a35′W67 form. The calculated IR spectra show fair agreement with the respective experimental spectra.

IR Spectra of $9MGW_1$ and $9MGW_2$ Probed through Red-Shifted UV Bands. Further discussion of the structures of these Gs monohydrates requires comparison with the result of 9MG. The IR spectrum probed through the -331 cm^{-1} peak in the UV spectrum of $9MGW_1$ (Figure 2b) is shown in Figure 6a. Similar IR spectra are observed with the UV laser fixed at the broad spectral region of 34 500 cm^{-1} of Figure 2b indicating that the UV spectrum in this region is due to a single monohydrated cluster. The symmetric and antisymmetric NH_2 stretches of this monohydrate are observed at 3420 and 3566 cm^{-1}, respectively, which are substantially lower than those obtained by probing the blue-shifted UV peaks described above. The frequencies of the observed IR transitions for the mono- and dihydrates of 9MG are listed in Table 2.

Besides the most stable monohydrate of the aW67 form, there are two other low-energy monohydrates, that is, those of the keto form (kW16) and the syn-enol form (sW16). The calculated IR spectra for these structures are also displayed in Figure 6a. They are apparently similar to each other except that the frequency corresponding to the free OH stretch of water is higher in the keto structure. The experimental IR spectrum exhibits a single transition of OH stretch at 3724 cm^{-1}, which favors the assignment of the keto hydrate. If the sW16 form is assumed, the corresponding frequency should be red-shifted with respect to that of the aW67 form (3711 cm^{-1}). A similar spectral shift of the OH stretch frequency is observed for the monohydrated clusters of the keto−enol tautomeric pair of 2′-pyridone and 2′-hydroxypyridine. For the keto monohydrate, IR transition of free OH stretch occurs at 3723 cm^{-1}, which is shifted by -9 cm^{-1} in the enol hydrate.[17] The symmetric and antisymmetric stretch frequencies of the NH_2 group are red-shifted from the respective transitions of the aW67 form (see Figure 5), which is also consistent with the calculated IR spectrum. The possibility of an imino-keto form for this monohydrate can be ruled out on the basis of the fact that the observed two transitions (3420 and 3566 cm^{-1}) are significantly higher than the frequencies of the N1H and N3H stretches reported for the imino forms of guanine (<3500 cm^{-1}).[18]

The assignment that the observed monohydrate is of the keto form appears to be inconsistent with the fact that UV features assignable to the corresponding monomer are apparently absent from the R2PI spectrum. For guanine, the amino-keto tautomers have not been detected by the R2PI technique[18] as opposed to the successful observation by IR spectroscopy in He droplets.[19] The failure to observe the biologically relevant tautomers has been ascribed to a substantial distortion of the amino group or to an ultrafast deactivation process to the ground state through a conical intersection.[20,21] Therefore, it is not unreasonable to expect that the missing amino-keto forms are observed by R2PI if they are less distorted in the excited state or if their ultrafast deactivation processes are suppressed upon hydration. This explanation is supported by the anomalous intensity distribution of the electronic spectrum shown in Figure 2b, which suggests that significant geometry changes still occur in the excited state. Likewise, the observation that the base pairs of guanine detected by R2PI are composed of the amino-keto tautomers[22,23] can be rationalized by a similar explanation.

We must also consider the possibility that the red-shifted UV spectrum observed at the $9MGW_1^+$ mass channel is due to fragmentation of larger hydrates. The IR spectrum of $9MGW_2$ obtained with the UV laser fixed at the same frequency (i.e., -331 cm^{-1}) is shown in Figure 6b. It appears that the transitions due to the two amino stretches are shifted upon addition of the second water molecule supporting the assignment that the red-shifted portion of the UV spectrum is that of the monohydrate. The most notable difference is that the antisymmetric NH_2 stretch transition at 3547 cm^{-1} is shifted by -19 cm^{-1} from that of the monohydrate. This dihydrate is assigned to the kWW16 structure (shown in part b) on the basis of its large stability of >22 kJ/mol with respect to other low-energy structures.[11] The calculated IR spectrum for the kWW16 structure is in reasonable agreement with the observed spectrum.

As shown in Figure 2a, spectral features corresponding to this monohydrate are absent in the spectrum in the $9MG^+$ mass channel, which implies that no fragmentation occurs upon ionization of this species. The absence of fragmentation may be rationalized by the specific ionization energetics of the keto forms. In the case of 2′-hydroxypyridine (enol), its hydrates were found to dissociate following one-color R2PI while those of 2′-pyridone could not be ionized by this method because of the high ionization energy relative to the two-photon excitation energy.[17]

IR Spectra of GsW_1 and GsW_2 Probed through Broad UV Bands. It is difficult to identify the mono- and dihydrate structures responsible for the broad UV spectrum since its severe spectral congestion precludes the use of UV−UV hole-burning spectroscopy. Instead, we have employed IR-UV hole-burning spectroscopy combined with theoretical calculations to obtain plausible hydration structures. Figure 7a shows the IR spectrum obtained with the UV laser fixed to the broad band of the Gs monohydrate indicated by an arrow in Figure 1b. Most of transitions appear to be broad, which suggests that the spectrum is composed of multiple structural isomers. However, transitions that can be assigned to the s35′W25′ and a35′W67 structures as described above (e.g., antisymmetric NH_2 stretch and free enol-OH stretch) are nearly absent from this spectrum. There-

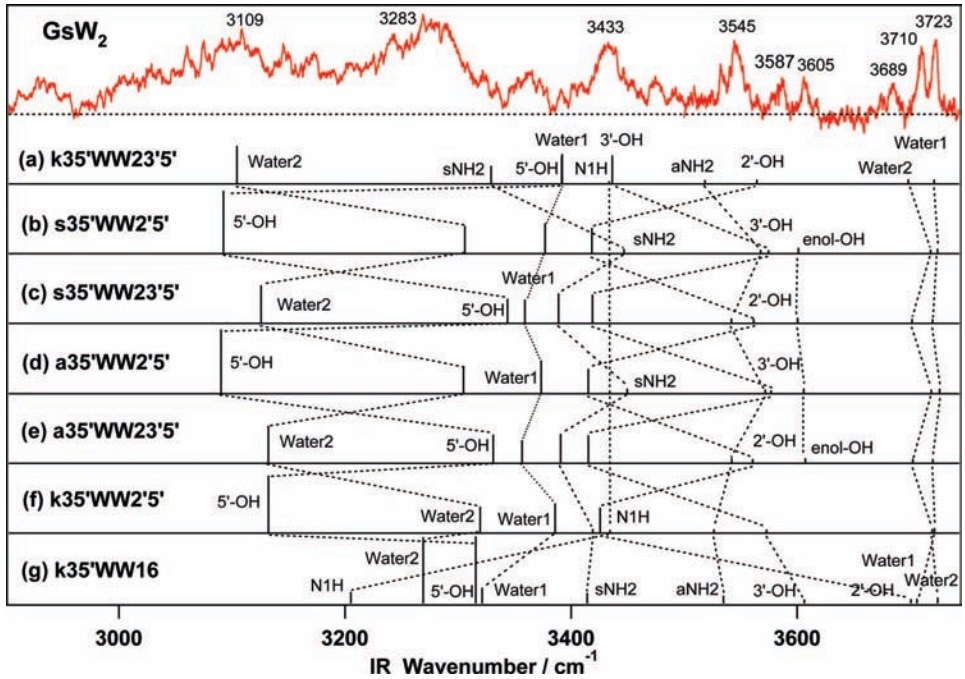

Figure 8. IR-UV double resonance spectrum of GsW_2 obtained by probing through the broad UV band indicated in Figure 1c. Calculated IR spectra (scaled by 0.957) are shown for the low-energy structures (a−g) of Figure 9.

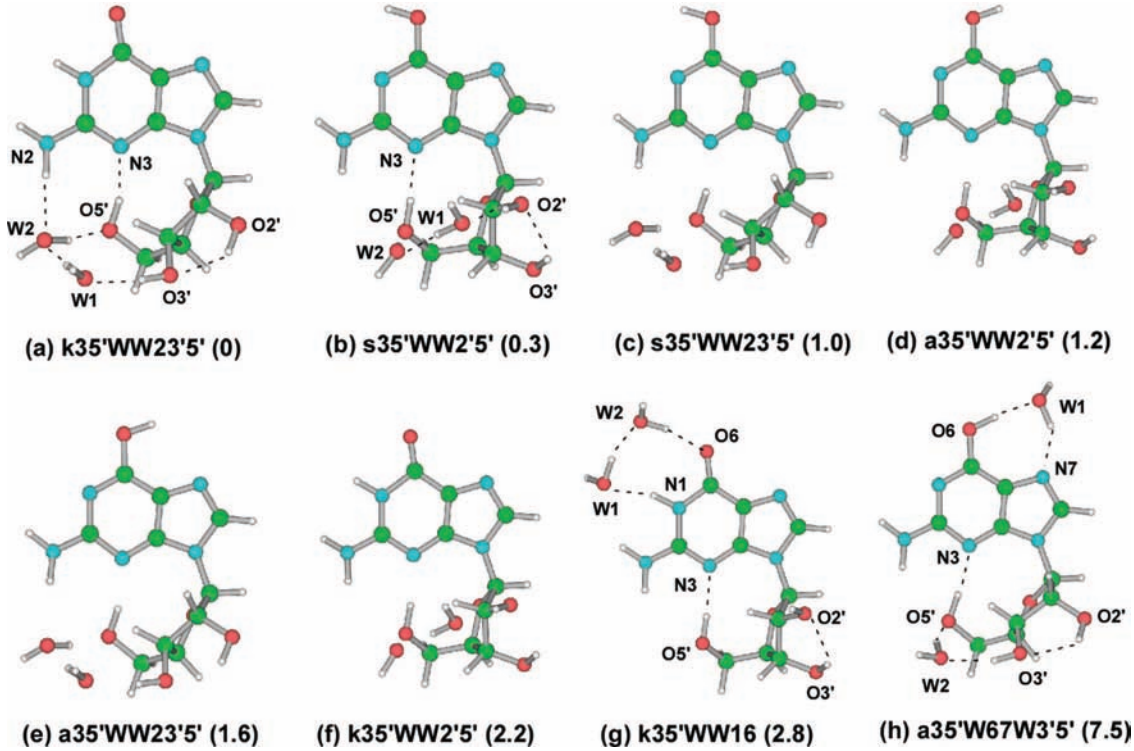

Figure 9. Low-energy dihydrate structures of Gs identified by the DFT/B3LYP calculation with a 6−311++G** basis set. The stabilization energies relative to the most stable structure of the respective dihydrates are shown in kJ/mol. The letters "s", "a", and "k" indicate tautomers with the guanine moiety in the syn-enol, antienol, and keto forms, respectively, while W refers to a water bridge and WW refers to a water dimer bridge. The numbers refer to the sites of the guanine moiety or to the sugar group linked by the water molecules.

fore, this IR spectrum should be assigned to other monohydrate clusters. The calculated IR spectra for the low-energy monohydrates with stabilization energies of <8 kJ/mol with respect to the most stable a35′W67 form (Figure 4) are displayed in Figure 7b−h. Most importantly, a pronounced transition that can be assigned to the free 2′-OH stretch of the sugar is observed at 3689 cm^{-1}. The persistence of the free 2′-OH stretch is consistent with the monohydrate structures of the W25′ and W16 forms shown in Figure 4. In addition, the free enol-OH stretch is absent from the spectrum, which suggests that the monohydrate spectrum is composed of the two keto structures, (b) k35′W25′ and (e) k35′W16. On the basis of this assignment, the broad transition around 3430 cm^{-1} may be associated with a composite of the symmetric NH_2 and N1H stretches of these

keto structures. Similarly, the doublet transition of the free OH of water (3711 and 3722 cm^{-1}) is explained by the coexistence of these monohydrates.

The UV spectrum measured at the GsW_2^+ mass channel shown in Figure 1c is apparently similar to the broad spectral region of GsW_1^+. The experimental IR spectrum of GsW_2 probed with the UV laser fixed at the same frequency is shown in the top panel of Figure 8. It appears to be similar to the corresponding spectrum of GsW_1 shown in Figure 7. However, there is a significant difference in the intensity of the transition because of the free 2′-OH stretch at 3689 cm^{-1}, which is less pronounced in the dihydrate spectrum of Figure 8. This implies that the 2′-OH group is influenced by the addition of the second water molecule. Also, broad transitions are observed around 3100 cm^{-1}, which are substantially red-shifted with respect to the monohydrate spectrum. These observations support that the broad UV band region of the monohydrate spectrum cannot be associated with the fragmentation of dihydrated clusters.

It was shown in our previous study[11] that several structural isomers of similar stability exist for the dihydrates of Gs. Most notable dihydrate structures correspond to those in which a water dimer links the 3′-OH and 5′-OH groups of the sugar and one of the water molecules is also hydrogen-bonded with the NH_2 group (labeled as WW23′5′). The guanine moiety is in the keto, syn-enol, or antienol form. Other dihydrate structures specific to Gs, in which the 2′-OH and 5′-OH groups of the sugar are bridged by a water dimer (WW2′5′), are found to be nearly isoenergetic to the WW23′5′ forms. The dihydrate of the keto form with a water dimer bridging the N1H and O6 sites (k35′WW16) is also as stable as these dihydrates. The lower energy structures with the relative stabilities of <10 kJ/mol are displayed in Figure 9.

The calculated IR spectra for these dihydrates are shown in Figure 8. The transition due to the 2′-OH stretch is shifted to a low frequency for the dihydrates a−f, which is consistent with the observation that the free 2′-OH transition appears to be weak. This frequency reduction is larger for the WW2′5′ structures, which is explained by the formation of strong hydrogen bonding of the 2′-OH with the water dimer. For the dihydrates of the WW23′5′ form, the red-shift of the 2′-OH transition is less significant. The result of structural calculation shown in Figure 9 reveals that internal hydrogen bonding is formed between the oxygen of the 3′-OH group and the hydrogen of the 2′-OH group. This hydrogen-bonding structure is different from that of the monohydrates shown in Figure 4 in which the hydrogen of the 3′-OH group is bonded to the oxygen of the 2′-OH group (except for the W3′5′ forms). It can be explained that such hydrogen bond switching is facilitated by the presence of the neighboring water dimer bridge. On the basis of these considerations, the dihydrate spectrum is assigned as arising from several structural isomers shown in Figure 9, which prevents unique assignment of the IR spectrum.

5. Concluding Remarks

The IR spectra of the mono- and dihydrated clusters of Gs have been recorded by the technique of IR-UV double resonance spectroscopy and have been compared with those of 9MG. The results are consistent with the previous structural assignments of the monohydrated clusters, which were obtained on the basis of the UV spectral signature and theoretical calculations. It is found that multiple structural isomers exist in both mono- and dihydrates of Gs and that the internal hydrogen-bonding structure of the Gs monomer is retained in all hydrates. The most stable monohydrate of Gs is observed to be that of the antienol form with water bridging O6H and N7, analogous to the case of the 9MG monohydrate. A monohydrate structure specific to Gs is also identified in which the guanine moiety is of the enol form and water bridges N2H of the guanine moiety and 5′-OH of the sugar. It is suggested that the broad UV spectrum of GsW_1 is due to other low-energy monohydrate structures of the amino-keto forms. The IR spectrum obtained for GsW_2 reveals that transition due to the free 2′-OH stretch is nearly absent, which indicates that this group is involved in the hydration. This could be responsible for the observation of significantly different UV spectra for the dihydrates of Gs and 2′-deoxyguanosine.[11] The present result also suggests that the amino-keto forms of Gs and 9MG, which are missing in the R2PI spectrum, can be observed upon hydration.

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