

Ultraviolet Spectroscopy and Theoretical Calculations of Mono- and Dihydrated Clusters of the Guanine Nucleosides: Possibility of Different Hydration Structures for Guanosine and 2′-Deoxyguanosine

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Mono- and dihydrated clusters of guanosine (Gs) and 2′-deoxyguanosine (2′-dGs) are formed by laser desorption combined with supersonic-jet cooling, and their electronic spectra are obtained by resonant two-photon ionization spectroscopy. The electronic spectra of these hydrated clusters appear to be composed of multiple structural isomers. Possible hydration structures are investigated based on comparison with the result for 9-methylguanine (9MG) and computational studies. The dihydrated cluster of Gs is found to exhibit absorption bands which are significantly shifted with respect to those of 2′-dGs and 9MG. The observation suggests the existence of specific dihydrate structures involving the 2′-hydroxy group of Gs.

Knowledge of the hydration of nucleic acids (NA) is essential for understanding the stabilization of their three-dimensional structures.[1] Results of crystallographic analyses of DNA by X-ray[2,3] and neutron diffraction[4] have demonstrated the presence of ordered hydration structures at the surface. This shell of tightly bound water molecules, mostly hydrated to the bases and phosphate groups,[5] is considered to be important for the reading of DNA sequences by recognition proteins. In contrast, hydration behavior around the backbone sugar groups has not been investigated extensively despite the fact that the conformational rigidity of RNA relative to DNA against humidity is often explained by the hydration of its 2′-hydroxy group.

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.[6,7] Nevertheless, reports on the formation of such hydrates are rather sparse due to the difficulty of volatizing thermally labile compounds. It has been shown that hydrated clusters of guanine can be formed by laser desorption followed by entrainment in a supersonic expansion.[8,9] In the present study, we have generated hydrated clusters of guanosine (Gs) and 2′-deoxyguanosine (2′-dGs) by the combination of laser desorption and supersonic jet-cooling. The structures and atom numbering of their canonical amino–keto forms are shown in Chart 1. Electronic spectra of the mono- and dihydrated clusters have been recorded by resonant two-photon ionization (R2PI) spectroscopy and compared with those of 9-methylguanine (9MG). The result suggests that the hydration structures of the two nucleosides are affected by the presence of the sugar group, which is discussed based on the results of ab initio calculations.

Theoretical

The DFT/B3LYP method with a 6-31++G(d,p) basis set was used for investigating stable geometries of the mono- and dihydrated clusters of Gs, 2′-dGs, and 9MG. Single-point calculations at the MP2/6-31++G(d,p) level were carried out for the lower energy structures to improve the accuracy of the

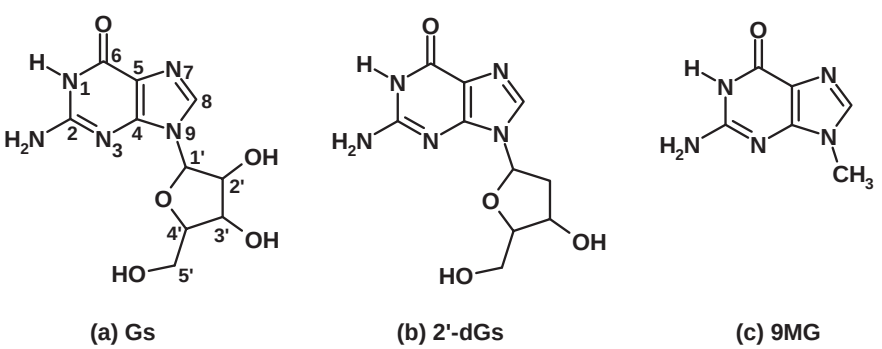

Chart 1. Structures of the amino–keto forms of (a) Gs, (b) 2′-dGs, and (c) 9MG. The atom numbering is shown in (a).

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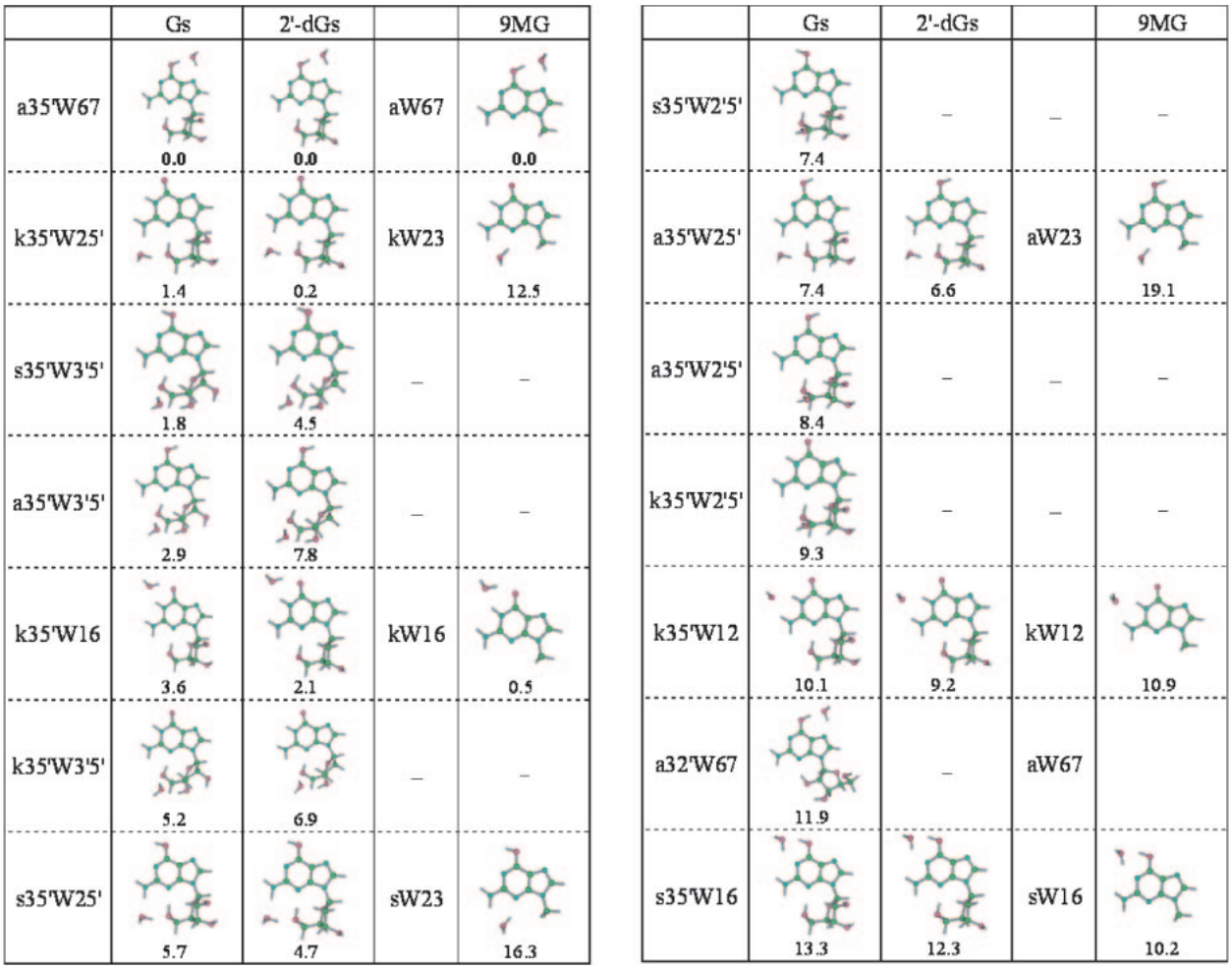


Figure 1. Stable monohydrate structures of Gs, 2′-dGs, and 9MG. The stabilization energies relative to the most stable structure of the respective monohydrates are shown in kJ mol^{-1}. The letters "s," "a," and "k" indicate tautomers with the guanine moiety in the *syn*-enol, *anti*-enol, and keto forms, respectively, while W refers to a water bridge. The numbers refer to the sites of the guanine moiety and/or the sugar group linked by the water molecule.

energetic ordering of the isomeric forms. Only energetically favorable amino tautomers were considered in this study. All calculations were performed using the Gaussian 03 program.[10]

Monohydrate Structures. For both Gs and 2′-dGs, the most stable structure of the monomer is based on their enol form of guanine with a strong internal hydrogen bond between 5′-OH of the sugar and N3 site of the guanine moiety.[11,12] Its OH group is oriented toward the N7 atom (*anti*-enol form, labeled as a35′) or toward the N1 atom (*syn*-enol form). Stable monohydrated structures obtained for Gs, 2′-dGs, and 9MG are summarized in Figure 1. The internal hydrogen-bonding conformation is retained in most of the stable monohydrates. The most stable monohydrate possesses a cyclic structure in which the water molecule bridges the O6H proton and the N7 site to form a seven-membered ring (a35′W67). This cyclic structure also corresponds to the lowest energy form of 9MG (aW67), which agrees with the result obtained at the MP2/aug-ccpvtz level.[13]

For the monohydrate of 9MG, the keto tautomer in which the water links the N1H and the O6 site (kW16) is as stable as the aW67 form. The corresponding hydrated structures in Gs and 2′-dGs (k35′W16) are also preferable. In addition to the hydration to the guanine moiety, cyclic structures in which water bridges N2H of the guanine moiety and 5′-OH of the sugar (k35′W25′, s35′W25′, and a35′W25′) are energetically low for Gs and 2′-dGs. Other cyclic structures linking the 3′-OH and 5′-OH groups of the sugar group by water (s35′W3′5′, a35′W3′5′, and k35′W3′5′) are also stable. No corresponding monohydrate structures exist in 9MG, and the cyclic structures directly linking the N2H and the N3 site (kW23, sW23, and aW23) are less stable than the aW67 form.

We have also studied monohydrate structures stabilized by an internal hydrogen bond between the 2′-OH of the sugar and the N3 site of the guanine, namely the anti form of Gs. The lowest energy structure corresponds to the *trans*-enol form with a W67 water bridge (a32′W67), which is less stable by 12 kJ mol^{-1} with respect to the most stable isomer. Other structures with extended conformations of the sugar group, in which no internal hydrogen bonding is retained, are also considerably higher in energy.

Dihydrate Structures. The low-energy structures and their relative stabilities for the dihydrated clusters of Gs, 2′-dGs, and 9MG are displayed in Figure 2. The most stable structures of Gs correspond to those in which a water dimer

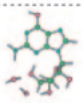
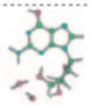
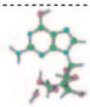
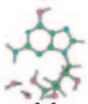
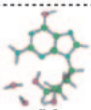
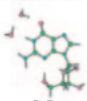
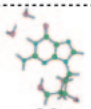
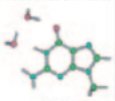
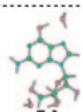
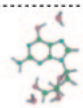
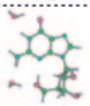
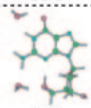
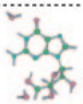
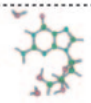
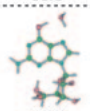
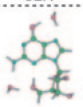
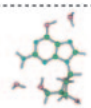
	Gs	2'-dGs		9MG
k35'WW23'5'	 0.0	 5.4	–	–
s35'WW2'5'	 0.3	–	–	–
s35'WW23'5'	 1.0	 7.2	–	–
a35'WW2'5'	 1.2	–	–	–
a35'WW23'5'	 1.6	 8.1	–	–
k35'WW2'5'	 2.2	–		
k35'WW16	 2.8	 0.0	kWW16	 0.0
a35'W67W3'5'	 7.5	 11.0	–	–
k35'W16W25'	 11.8	 9.0	kW16W23	 22.3
k35'W16W3'5'	 11.8	 14.2	–	–
a35'W2'5'W67	 12.7	–	–	–
a35'W25'W67	 13.0	 11.1	aW23W67	 24.3

Figure 2. Stable dihydrate structures of Gs, 2′-dGs, and 9MG. The stabilization energies relative to the most stable structure of the respective dihydrates are shown in kJ mol^{-1}. The letters "s," "a," and "k" indicate tautomers with the guanine moiety in the *syn*-enol, *anti*-enol, and keto forms, respectively, while W refers to a water bridge and WW to a water dimer bridge. The numbers refer to the sites of the guanine moiety and/or the sugar group linked by the water molecule(s).

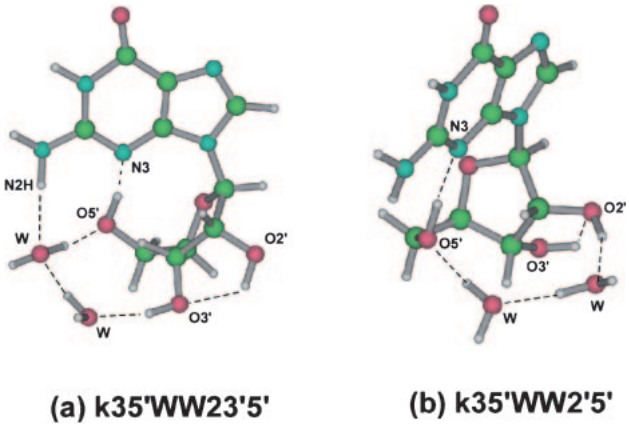


Figure 3. Expanded view of the specific dihydrate structures of Gs, (a) k35′WW23′5′ and (b) k35′WW2′5′, shown in Figure 2. In both cases, the internal hydrogen bonding between the 5′-OH group of the sugar ring and the N3 atom of the guanine is retained. Similar dihydrate structures with the guanine moiety in the enol forms are as stable as these keto structures.

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 *anti*-enol form. Similar hydration structures in which the 2′-OH and 5′-OH groups of the sugar are bridged by a water dimer (WW2′5′) are also found to be stable. The dihydrate in which the guanine moiety is in the keto form and the two water molecules form a dimer and bridge the oxygen atom of the C=O group and the N1H site (k35′WW16) is nearly isoenergetic to these dihydrates. The relative stabilities of these stable dihydrate structures are consistent with the result obtained by full geometry optimization at the MP2/6-31++G(d,p) level. The specific dihydrate structures of Gs involving the sugar group, k35′WW23′5′ and k35′WW2′5′, are shown in an expanded scale in Figure 3.

There is a substantial difference in the dihydrate structures of Gs and 2′-dGs. The lowest energy dihydrate structure of 2′-dGs is found to be that of the k35′WW16 form. The dihydrate structures of the cyclic forms WW23′5′ and WW2′5′ are calculated to be less stable by >5 kJ mol^{-1} with respect to the lowest energy structure. The lowest energy dihydrate structure of 9MG corresponds to the kWW16 form, analogous to case of 2′-dGs. This water dimer structure is consistent with the results for 9H-guanine.[14,15] All other dihydrated structures of 9MG are less stable by >22 kJ mol^{-1}.

Experimental

The pulsed laser desorption source employed in this study has been described elsewhere.[16,17] Laser desorption was accomplished by irradiating a sample pellet placed inside the source by the second harmonics of a YAG laser. The plume of desorbed molecules was directed through a narrow channel (1 mm in diameter and 10 mm in length) with Ar carrier gas at 5 atm. The advantage of this desorption source over other laser desorption sources is that efficient hydration of desorbed molecules occurs in the channel region by multiple collisions with water molecules. The sample pellet was prepared by mixing sample powder with graphite

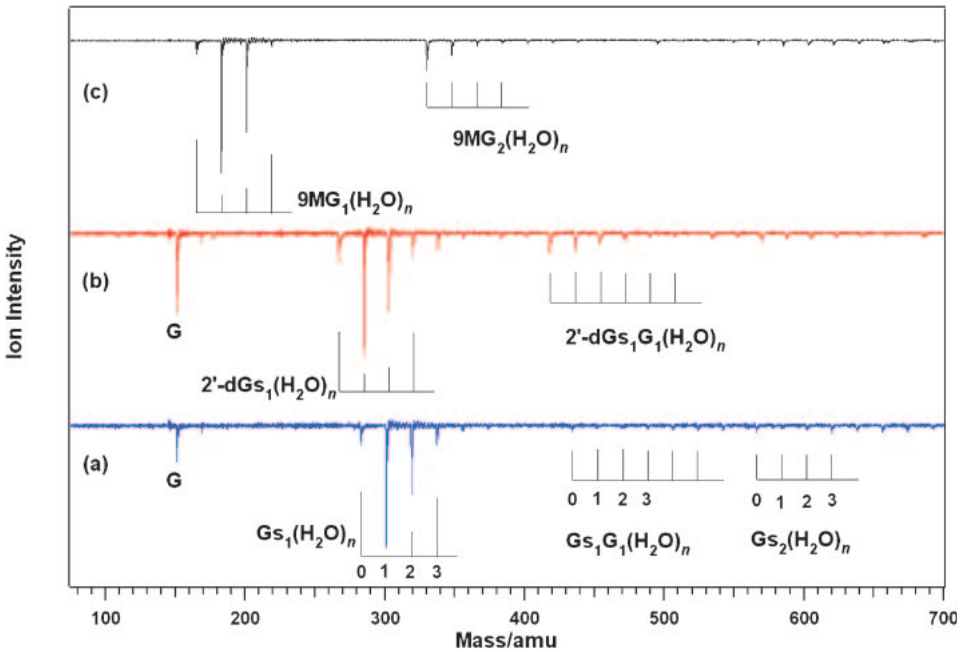


Figure 4. Typical TOF mass spectra showing peaks of hydrated clusters of (a) Gs, (b) 2′-dGs, and (c) 9MG. The mass peak labeled G in parts (a) and (b) corresponds to guanine monomers produced upon laser desorption. In all spectra, R2PI was carried out at 285 nm.

(5%) and pressing with a hydraulic press. Water vapor was introduced by passing the Ar carrier gas through a reservoir. The desorption laser was operated with typical fluences of <5 mJ pulse^{-1}, and focused to a spot size of 2 mm. Mass-selected R2PI spectra were obtained by an experimental setup that has been described previously.[16]

Results

Figure 4 shows typical TOF mass spectra of hydrated clusters of Gs, 2-dGs, and 9MG obtained following R2PI. The spectrum for Gs is dominated by mass peaks of the monohydrate ion $Gs(H_2O)_1^+$ and dihydrate ion $Gs(H_2O)_2^+$ while larger hydrates are less abundant. A mass peak corresponding to guanine monomer ion ($m/z = 151$) arises from decomposition upon laser desorption. The appearance of these hydrated clusters in the mass spectrum is in striking contrast with the case of unsubstituted guanine, in which mass peaks of the monomer hydrates are observed to be very weak.[16]

Guanosine. R2PI spectra obtained at the mass channels of Gs^+, $Gs(H_2O)_1^+$, and $Gs(H_2O)_2^+$ are displayed in Figure 5. The bottom spectrum reveals sharp peaks around 34500 cm^{-1}, which match those in the spectrum of Gs monomer reported by Nir et al.[11,12] The low-frequency modes built on the origin band (marked by an asterisk in Figure 5a) 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.

The R2PI spectrum of $Gs(H_2O)_1^+$ in Figure 5b is likely to be composed of multiple structural isomers. A series of narrow bands appearing in the energy region below <34200 cm^{-1} correspond to the spectral features of one isomer. The red-most peak located at -653 cm^{-1} relative to that of the monomer

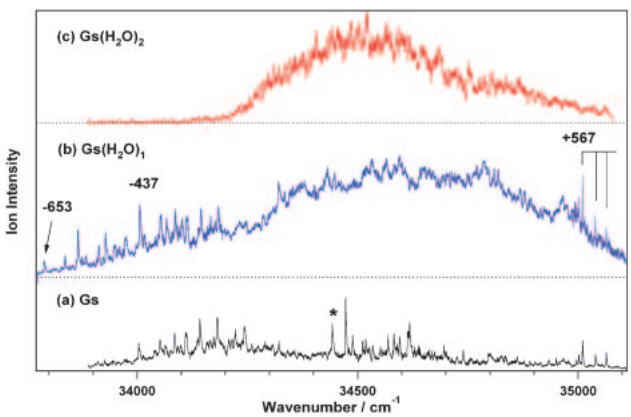


Figure 5. R2PI spectra obtained at the mass channels of (a) Gs^+, (b) $Gs(H_2O)_1^+$, and (c) $Gs(H_2O)_2^+$. The spectra are not normalized to the laser intensity. The asterisk in (a) indicates the origin band of the Gs monomer. The spectral shifts (cm^{-1}) for the pronounced peaks of the monohydrates from this origin band are indicated in (b).

origin is followed by a number of peaks at irregular frequency intervals. Broad bands with no prominent peaks appearing in the region between 34200 and 35000 cm^{-1} may be associated with a second isomer. Beyond this energy region, a couple of sharp features are noticeable and the most prominent peak is blue-shifted by 567 cm^{-1} from the band origin of Gs monomer.

The R2PI spectrum was measured in the Gs^+ mass channel (Figure 5a) with water entrained in the Ar carrier gas. It exhibits additional sharp bands (e.g., a peak located at -437 cm^{-1}) which match those observed in the $Gs(H_2O)_1^+$ mass channel. These observations indicate that this monohydrate undergoes evaporation of the water molecule upon ionization when

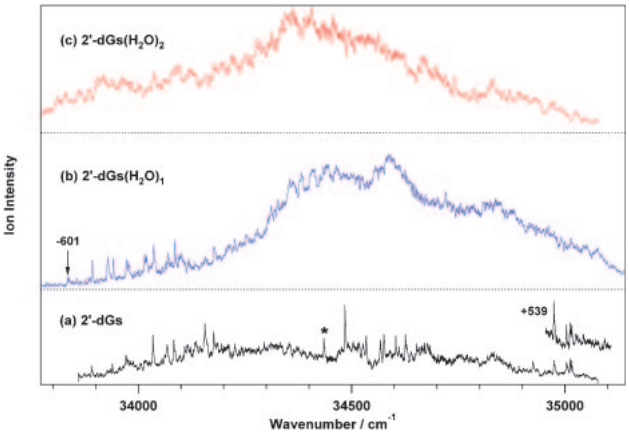


Figure 6. R2PI spectra obtained at the mass channels of (a) 2′-dGs$^+$, (b) 2′-dGs$(H_2O)_1{}^+$, and (c) 2′-dGs$(H_2O)_2{}^+$. The spectra are not normalized to the laser intensity. The asterisk in (a) indicates the origin band of the 2′-dGs monomer. The spectral shifts (cm^{-1}) with respect to this monomer origin are indicated for the pronounced peaks assigned to the monohydrates in (a) and (b).

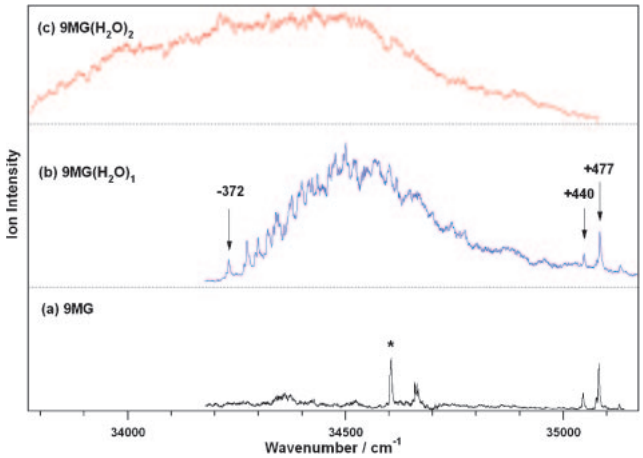


Figure 7. R2PI spectra obtained at the mass channels of (a) 9MG$^+$, (b) 9MG$(H_2O)_1{}^+$, and (c) 9MG$(H_2O)_2{}^+$. The spectra are not normalized to the laser intensity. The asterisk shown in (a) indicates the origin band of the 9MG monomer, and the spectral shifts (cm^{-1}) of the monohydrate peaks with respect to this origin are indicated in (b).

excited through this energy region. Its efficiency appears to be independent of the pulse energy of the ionization laser. In contrast, the broad bands observed in the 34500 cm^{-1} region of the Gs$(H_2O)_1{}^+$ spectrum are nearly absent from the spectrum measured at the Gs$^+$ ion channel. Since the broad spectrum could also be attributed to that arising from fragmentation of larger hydrated clusters, e.g., Gs$(H_2O)_2$, R2PI scans have been performed at very low concentrations of water to decrease their abundances in the jet. Nevertheless, a similar broad spectrum of Gs$(H_2O)_1{}^+$ can be observed under these conditions and thus it is likely to be assigned to a third isomer of the monohydrate. It is therefore suggested that the monohydrate spectrum is composed of at least three different structural isomers.

The R2PI spectrum obtained at the Gs$(H_2O)_2{}^+$ ion channel in Figure 5c is significantly different from that of Gs$(H_2O)_1{}^+$. It consists only of broad features with no significant intensity in the energy region below 34200 cm^{-1}. Although the broad features are apparently similar to those observed in the monohydrate spectrum, we rule out the possibility of fragmentation of larger hydrates into this Gs$(H_2O)_1{}^+$ ion based on the absence of noticeable dependence of the relative intensity on the water concentration.

2′-Deoxyguanosine. Figure 6 shows R2PI scans performed by detecting 2′-dGs$^+$, 2′-dGs$(H_2O)_1{}^+$, and 2′-dGs$(H_2O)_2{}^+$ ions. The spectrum of 2′-dGs$(H_2O)_1{}^+$ in Figure 6b exhibits sharp peaks in the energy region below 34200 cm^{-1}. The observed red-most peak is red-shifted by 601 cm^{-1} from the origin band of the 2′-dGs monomer (marked by an asterisk in Figure 6a). It also gives rise to broad features in the vicinity of the monomer origin, which appear to be more pronounced than in the spectrum of Gs$(H_2O)_1$.

In contrast to the case of Gs, the spectrum obtained by detecting 2′-dGs$(H_2O)_1{}^+$ ions reveals no resolved structure to the blue of the monomer origin. The R2PI scan detected at the 2′-dGs$^+$ ion channel shown in Figure 6a exhibits sharp peaks in the energy region of 34000 cm^{-1}. They correspond well to those of the monomer spectrum. Additional resolved spectral features are noticeable around 35000 cm^{-1} (e.g., a peak located at +539 cm^{-1} from the monomer origin). Since these peaks cannot be detected in the absence of water vapor, they are assigned to those of a monohydrated cluster of 2′-dGs. Thus, the monohydrate cluster is likely to undergo efficient fragmentation upon excitation and subsequent ionization at this energy region.

9-Methylguanine. Figure 7 shows the R2PI spectra obtained at the mass channels of 9MG$(H_2O)_1{}^+$ and 9MG$(H_2O)_2{}^+$ ions. The bottom spectrum is obtained by measuring the signal of 9MG$^+$ monomer ions in the presence of water vapor. The prominent peak of the 9MG monomer, marked with an asterisk in Figure 7a, was assigned to the origin of the enol form with its hydroxy group oriented toward the N7 atom (i.e., *anti*-enol form). The spectrum in Figure 7b reveals a couple of prominent peaks at +440 and +477 cm^{-1} to the blue of the monomer origin, consistent with a previous report by Chin et al.[13] They identified that in this monohydrate the *anti*-enol form is retained upon hydration with the water molecule bridging the O6H proton and the N7 site (aW67 structure in Figure 1). This monohydrate corresponds to the most stable monohydrate structure of 9MG.

As shown in Figure 7b, additional resolved spectral features are observed to the red of the monomer origin, which cannot be recognized in the spectrum reported by Chin et al.[13] These red-shifted peaks are accompanied by underlying broad background features. The red-most peak at 34232 cm^{-1} is red-shifted by 372 cm^{-1} from the monomer origin. The anomalous intensity distribution and irregular vibrational intervals are indicative of large geometry changes induced by the electronic excitation.

The spectrum obtained by measuring monomer ions of 9MG$^+$ exhibits sharp peaks which match closely the blue-shifted features appearing in the spectrum of Figure 7b. This indicates the loss of the water molecule upon ionization for this hydrate, which is consistent with previous result.[13] In

contrast, the red-shifted features observed at the $9MG(H_2O)_1^+$ mass channel are entirely absent from the spectrum of $9MG^+$. It could also be explained that fragmentation of larger clusters upon ionization is responsible for the observation of the red-shifted spectrum. However, the results obtained by decreasing the water vapor entrained in the Ar carrier gas show no significant difference in the intensity of the broad spectrum with respect to that of the sharp features. Therefore, we suggest that contribution of fragmentation of larger clusters to the $9MG(H_2O)_1^+$ ion signal is negligible or minor, and the broad spectrum is due to the corresponding neutral species.

The R2PI spectrum detected at the $9MG(H_2O)_2^+$ mass channel in Figure 7c is further red-shifted from that of the monohydrate and reveals only broad bands. It reveals only broad band features and resembles that of $2'\text{-dGs}(H_2O)_2^+$.

Discussion

Structural assignment for hydrated clusters of small molecules is usually performed by the separation and identification of their electronic spectra using double-resonance spectroscopic methods.[6,7] However, application of these methods to more complicated systems such as nucleoside molecules could be hampered by the possible existence of multiple low-energy structural isomers. In this section, hydration structures of Gs and 2′-dGs are discussed qualitatively based on comparison with the result of 9MG, in which the sugar group of the nucleosides is replaced with a methyl group.

Possible Monohydrate Structures. The monohydrate structure of 9MG which gives rise to the blue-shifted spectrum was identified as the most stable aW67 form based on the results of IR–UV double resonance measurements and theoretical calculations.[13] As shown in Figure 1, the monohydrate of the keto form kW16 is calculated to be as stable as the aW67 form and thus expected to be abundant in a supersonic expansion. Nevertheless, spectral features due to the keto forms of 9MG and its monohydrate have not been detected in the respective R2PI spectra. This puzzling observation was associated with the occurrence of specific ultrafast excited-state dynamics, which preclude sufficient ionization.[13] Other monohydrated structures such as the *syn*-enol form, in which the water bridges the N1 nitrogen atom of guanine and the OH proton pointing toward the N1 site (sW16), are less stable by $>12\,\mathrm{kJ\,mol^{-1}}$.

The electronic spectra of $9MG(H_2O)_1$ obtained in the present study reveal additional spectral features which are red-shifted from that of the monomer. These peaks are apparently absent from the spectrum reported by Chin et al.[13] although broad features are noticeable in the vicinity of the monomer origin. The newly observed spectrum can be attributed to the existence of a second isomer, in addition to the lowest energy *anti*-enol isomer (aW67) observed in the blue-shifted region. Since the monohydrates of the keto form (kW16) and the enol form (aW67) are nearly isoenergetic (Figure 1) and all other structures are less stable, the red-shifted portion of the monohydrate spectrum could be associated with this keto form.

For unsubstituted 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] More importantly, the keto forms observed in the R2PI spectra have been identified as those of the imino tautomers. The failure to observe the biologically relevant tautomers has been ascribed to a substantial distortion of the amino group or an ultrafast deactivation process to the ground state through a conical intersection.[20,21] Since the imino tautomers of unsubstituted guanine are nearly absent in the R2PI spectra obtained with the desorption method employed in this study,[17] we rule out the possibility that the newly observed monohydrate of 9MG is one of the imino–keto forms. The hydrates of the amino–keto forms could be observed by R2PI if they are less distorted in the excited state or their ultrafast deactivation processes are suppressed upon hydration. The anomalous intensity distribution is consistent with large geometry changes induced by the electronic excitation.

Next, we must explain the apparent result that no fragmentation occurs upon ionization of the keto form (kW16) while the enol form (aW67) can be observed in the $9MG^+$ channel by a loss of the water molecule. In the case of the hydrated clusters of nucleobase mimic molecules, 2-pyridone and 2-hydroxypyridine, the hydrates of its enol form (2-hydroxypyridine) were found to dissociate following one-color R2PI by Zwier's group.[22] In contrast, 2-pyridone monomer and its hydrates cannot be ionized by this method due to a higher ionization energy than in the enol form.[23] The absence of fragmentation in the red-shifted monohydrate of 9MG may be rationalized by similar ionization energetics.

It is not clear why the red-shifted spectrum is observed in this study in addition to the previously reported blue-shifted bands. One possible reason for this discrepancy can be attributed to the desorption source employed in this study, which is more effective in increasing the abundances of hydrated clusters.[16,17] This difference can be seen in Figure 7a, wherein the relative abundance of the monohydrate fragment of 9MG to its monomer appears to be higher than that reported by Chin et al.[13] There is also a possibility that the monohydrate cluster of the kW16 form could have a reduced excited-state lifetime with respect to that of the enol form, analogous to the case of guanine.[20,21] In this case, its ionization efficiency may be sensitive to the pulse energy of the ionization laser.

The R2PI spectrum obtained at the mass channel of $Gs(H_2O)_1^+$ in Figure 5b reveals a couple of sharp peaks to the blue of the monomer origin. On the basis of the similarity to that of 9MG, this monohydrate may be assigned to the most stable structure with the guanine moiety in the *anti*-enol form (a35′W67). It is important to note that the spectral shift from the monomer origin ($+567\,\mathrm{cm^{-1}}$) is larger than in 9MG ($+440\,\mathrm{cm^{-1}}$). The larger spectral shift for Gs indicates that the internal hydrogen bonding structure between the 5′-OH group and the N3 site of the guanine moiety is less stable in the excited state. However, the frequency intervals of the main peaks appear to be different from those of 9MG. The origin of the low-frequency features for the monohydrate of 9MG has not been identified, but could be linked to torsional motion of the methyl group. The corresponding low-frequency motions of Gs are considered to be the relative motions of the sugar and guanine moieties, which can be seen in the monomer spectrum.[11] Therefore, the observed spectral pattern may be explained by the difference in the vibrational frequencies of the two low-frequency motions.

The monohydrate spectrum of Gs also reveals sharp peaks in the lower energy region, suggesting the existence of a second structural isomer of the monohydrate. Their spectral shifts from the monomer origin are apparently larger than those in the monohydrate spectrum of 9MG. Furthermore, these peaks can be observed in the spectrum obtained by detecting at the Gs^+ mass channel as a result of fragmentation upon ionization. In contrast, the lower energy features of the 9MG monohydrate are absent from the monomer spectrum. On these bases, we conclude that this spectrum does not correspond to the red-shifted spectrum of 9MG. In both monohydrate structures of Gs and 2′-dGs, the cyclic structures in which the water bridges N2H of the guanine moiety and 5′-OH of the sugar (k35′W25′, s35′W25′, and a35′W25′) are energetically as low as the most stable *anti*-enol form (a35′W67). Therefore, the red-shifted spectrum could be associated with one of these monohydrates. Furthermore, the observation of this monohydrate in the Gs^+ ion channel as a result of fragmentation suggests that this hydrate is either enol form (s35′W25′ or a35′W25′).

It should be noticed in the monohydrate spectrum of Gs (Figure 5b) that broad band features appear between the two spectral regions described above. Similar, but more pronounced broad features are also observed in the corresponding spectrum of 2′-dGs shown in Figure 6b. These broad band features cannot be associated with fragmentation of larger hydrated clusters and thus assigned to those of the monohydrate. Furthermore, they cannot be observed in the spectra measured at the respective monomer ion channel. These observations suggest that the broad spectrum corresponds to the red-shifted spectrum of $9MG(H_2O)_1$.

The monohydrate spectrum of 2′-dGs recorded under the same experimental conditions gives no resolved features in the blue-shifted region (Figure 6b). Instead, a couple of sharp peaks assignable to a monohydrate cluster are observed in the spectrum of $2'\text{-dGs}^+$, which implies that this monohydrate undergoes complete fragmentation upon ionization. The different fragmentation behavior for Gs and 2′-dGs cannot be explained readily by the stability of the monohydrated clusters shown in Figure 1. The monohydrate spectrum also gives rise to sharp bands to the red of the monomer origin. These peaks correspond well to those of the Gs monohydrate, which suggests that this monohydrate is of either enol form, s35′W25′ or a35′W25′. The broad spectral features observed in the vicinity of the monomer origin are more pronounced for the monohydrate of 2′-dG. It is difficult to rationalize this difference based on the theoretical result that the relative stability of the keto form (k35′W16) of 2′-dGs with respect to the most stable form (a35′W67) is nearly the same as that of Gs (Figure 1).

Specific Dihydrate Structure of Guanosine. The R2PI spectrum for dihydrated 9MG shown in Figure 7c appears to be more red-shifted than the monohydrate spectrum and exhibits no sharp features. Theoretical calculations on dihydrated clusters of guanine[14,15] indicated that the lowest energy structure corresponds to that of the 9H-keto form, in which two water molecules form a dimer and bridge the oxygen atom of the C=O group and the N1H site. This water dimer motif explains the result for 9MG. As shown in Figure 2, the lowest energy structure corresponds to that of keto form (kWW16) and all other dihydrated structures are less stable by >18 kJ mol^{-1}. It is therefore suggested that the observed spectrum of the dihydrate is that of the kWW16 form. The assignment of the keto form is consistent with the absence of this transition in the $9MG(H_2O)_1{}^+$ channel, i.e., no evidence of fragmentation, and with a larger red-shift than the monohydrate spectrum.

The spectrum of $Gs(H_2O)_2{}^+$ is remarkably different from that of $9MG(H_2O)_2{}^+$. It consists only of broad features located in the vicinity of the monomer spectrum, with no discernible features corresponding to the red-shifted region of the monohydrate spectrum. The absence of red-shifted features suggests that this dihydrate does not correspond to the most stable form of 9MG (kWW16). According to the result in Figure 2, three dihydrate structures of Gs in which a water dimer links the 2′-OH and 5′-OH groups of the sugar and forms a hydration shell (s35′WW2′5′, a35′WW2′5′, and k35′WW2′5′) are found be stable. These structures are specific to the dihydrate of Gs, which could be associated with the observation that the dihydrate spectra of Gs and 2′-dGs differ from each other. In contrast, the dihydrate spectrum of 2′-dGs resembles that of 9MG, which is consistent with the prediction that the most stable dihydrate structure of 2′-dGs is of the k35′WW16 form.

Conclusion

Electronic spectra of hydrated clusters of Gs and 2′-dGs have been recorded by R2PI spectroscopy and compared with those of 9MG. In both nucleosides, the monohydrate spectra are assigned as a composite of multiple structural isomers. Part of the spectra is found to be similar to that of 9MG, which suggests that the hydration occurs around the guanine moiety. Distinct resolved spectral features observed to the red of the monomer origin band are associated with the formation of specific hydration structures involving the sugar group. A theoretical result reveals two types of such monohydrates. In one, water bridges between the NH_2 group of the guanine moiety and the 5′-OH group of the sugar and, in the other, the 3′-OH and 5′-OH groups are linked by water. The R2PI spectra for the dihydrated clusters of Gs and 2′-dGs are found to be significantly different from each other. The dihydrate spectrum of Gs is blue-shifted with respect to those of 2′-dGs and 9MG. Structural calculation suggests the existence of a specific dihydrate structure involving the sugar group of Gs. The separation and identification of the electronic spectra of these hydrates based on double-resonance spectroscopic measurements will enable us to obtain more detailed hydration structures.

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