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Microhydration of Guanine Base Pairs

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Water plays an important role in the structure and function of DNA and RNA. Recent theoretical calculations show that for the AT base pair, the presence of two water molecules suffices to stabilize a stacked structure more than a hydrogen-bonded structure.[1,2] The enhanced stabilization results from the water molecules surrounding the base pair and creating a bridge between the bases. Calculations predict that for GG base pairs about 20% of the structures will stack upon addition of two water molecules.[1] Additional water further stabilizes stacking structures, and upon addition of a fourth water molecule 90% of the structures are predicted to be stacked. Sivanesan et al. predict that five or six water molecules preferentially stabilize GC pairs.[3] Microhydration also can stabilize specific tautomers.[4] In solution, bulk solvent hides the details of these interactions, but by isolating the bases and sequentially adding single solvent molecules it is possible to study the changes caused by microscopic solvent effects on base interactions.

Experimental data on clusters of base pairs with water are hard to obtain because of the difficulties of producing these clusters in the gas phase. Kim et al. measured ionization potentials for hydrated adenine and thymine, which decrease with the third water molecule.[5] Kang et al. studied femtosecond photodissociation of hydrated adenine,[6] finding a 200 fs lifetime for the excited state, ascribed to dissociation. For hydrated methyl-substituted uracil and thymine monomers, He et al. found an excited state with nanosecond lifetimes, which decrease with increasing number of water molecules.[7] Piuzi et al. observed three isomers for guanine monomer (G) with one water without determining their structure,[8] and Chin et al. recently reported that 9-methylguanine monomer with one water retains its monomeric enol form.[9] Here we present the first UV and IR spectroscopy of a base pair—the guanine dimer, GG—with one and two water molecules. We recorded the vibronic spectra of the mass-selected $GG(H_2O)$ and $GG(H_2O)_2$ clusters using resonant two photon ionization (R2PI). We used IR−UV double resonance spectroscopy to obtain IR spectra of the ground state in the region 3150−3850 cm^{-1} encompassing the OH, NH, and NH2 stretch frequencies. Shifts in these frequencies as a result of H-bonding allow us to assign cluster structures.

We produce clusters by laser desorbing a mixture of guanine and ice from a graphite substrate, followed by entrainment in a supersonic expansion. We perform R2PI in the source of a time-of-flight mass spectrometer. For double resonant spectroscopy, the R2PI signal at a fixed wavelength serves as a probe, following either a UV or an IR "burn" laser pulse. When the burn laser scans over a resonance it causes depletion of the ground state, which leads to a decrease in the ion signal from the probe laser, provided both lasers are resonant with the same isomer.

Figure 1 shows a comparison between the R2PI spectra of GG, $GG(H_2O)$, and $GG(H_2O)_2$ clusters. Spectrum (a) is the R2PI spectrum of the GG dimer.[10] UV−UV double resonance spectroscopy reveals two different structures, GG1 and GG2.[11] Spectrum (b) in Figure 1 is the R2PI spectrum of $GG(H_2O)$. The origin is red-shifted by 67 cm^{-1} compared to the origin of GG1. For the hydrated clusters we did not obtain UV−UV double resonance spectra with a sufficient signal-to-noise ratio. For $GG(H_2O)$ there appears to be a broad spectrum underlying the structured spectrum, possibly due to another isomer and complicating the hole burning. However, we did determine that all the lines in this spectrum belong to one single isomer, based on IR−UV double resonance, as detailed below. Spectrum (c) is the R2PI of the cluster $GG(H_2O)_2$. This spectrum is noticeably broader compared to the spectrum of GG and $GG(H_2O)$. The farthest red-shifted peak that is also the most intense peak in the spectrum is red-shifted by 177 cm^{-1} compared to the origin of GG1. Here, too, IR−UV double resonance reveals one single isomer. Upon careful comparison of the $GG(H_2O)$ and GG1 spectra, it is apparent they exhibit very similar vibronic spectra and that the $GG(H_2O)$ spectrum shows no major peaks that would correspond to the GG2 spectrum. This suggests that the water molecule is attached to GG1 clusters and not to GG2 clusters. IR spectra can further elucidate the structures.

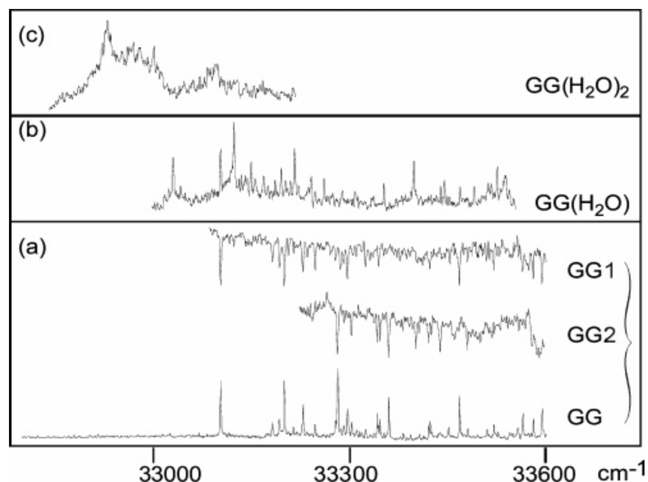

Figure 1. R2PI spectra. (a) Guanine dimers GG. Traces GG1 and GG2 are UV−UV double resonance spectra of GG. (b) GG with one water. (c) GG with two waters.

Figure 2 represents the IR−UV double resonance spectra of GG, $GG(H_2O)$, and $GG(H_2O)_2$. For the GG dimer we have previously assigned two structures (GG1 and GG2) based on comparison of the IR−UV spectra of each isomer with ab initio calculations.[11] For the hydrated clusters, we measured separate IR spectra with the probe laser tuned to each UV peak. We obtained the same IR spectrum for each UV peak, which shows that only one isomer is present both in the $GG(H_2O)$ and in the $GG(H_2O)_2$ R2PI spectrum. Our structure analysis of the hydrated dimers is based on comparison of their IR frequencies with those of the nonhydrated dimers.

The IR spectra of $GG(H_2O)$ and $GG(H_2O)_2$ are similar to that of GG1, characterized by hydrogen-bonding interaction between the two bases. This interaction leads to an IR spectrum distinct from that of GG2 because of the different modes involved (see

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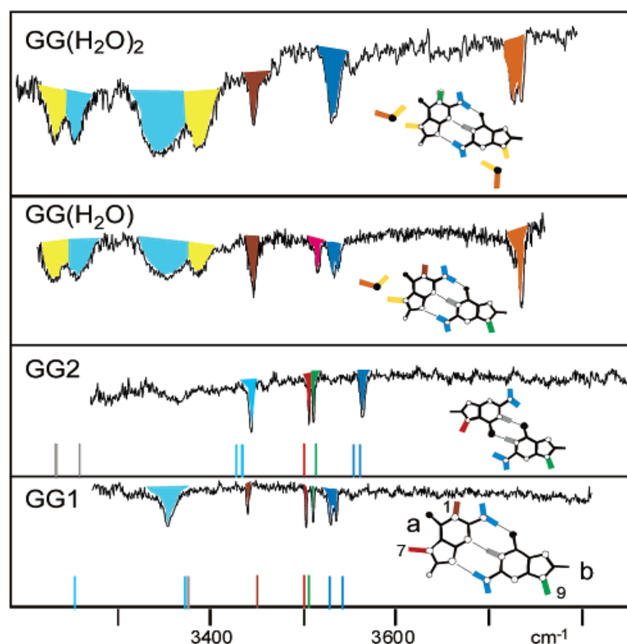

Figure 2. IR−UV double resonance spectra of GG structures GG1 and GG2 and of the dimers with one and two water molecules. The stick spectra are calculated frequencies for the respective structures without water. Color coding indicates the respective modes as indicated in the structures.

Figure 2). The hydrogen bonding between the GG dimer and water further modifies the spectrum, providing clues about the position of the water molecules. In the IR spectra of GG(H_2O) we observe a strong and sharp peak at 3735 cm^{-1}, which is typical for a free OH of water. The N7H(a) stretching mode (indicated in red and with "(a)" referring to a particular guanine as labeled in Figure 2) that appears in GG1 at 3504 cm^{-1} is absent in GG(H_2O). This is an indication that the N7 hydrogen is involved in hydrogen bonding. In that case one would expect a red-shift of several hundred cm^{-1} accompanied by peak broadening. In fact, we see four broad peaks below 3400 cm^{-1}. We assign two of those, marked in light blue, to symmetric NH_2 stretches, which for the bare GG dimer are calculated to be at 3253 and 3374 cm^{-1} for G_a and G_b, respectively.[11] This is in very good agreement with the experimental values in the hydrated spectra; in the GG1 spectrum the 3374 cm^{-1} is in good agreement, while the second peak is outside the frequency range covered in this spectrum. We assign the other two peaks, marked in yellow, to the shifted N7H stretch and to the bound water OH, which forms a hydrogen bond with the carbonyl oxygen of G_a. Without further calculation we cannot decide which of these two is which.

Figure 2 also shows the IR spectrum of GG(H_2O)$_2$. In this spectrum we observe a doublet at 3735/3727 cm^{-1} corresponding to the individual free OH stretching vibrations of the two water molecules. The N9H vibration of G_b (marked in green) is missing from its original position in GG1, which indicates that the second water is hydrogen-bonded to this position (see Figure 2). This interpretation implies two additional broad red-shifted peaks due to the N9H of G_b and the bound OH of the second water, hydrogen-bonded to the G_b(NH_2). However, since these peaks are broad it is plausible that they overlap with the other broad peaks below 3400 cm^{-1} and cannot be resolved. This may account for the increased intensity of the peaks below 3400 cm^{-1}. It is important to point out that determination of the order in which the water molecules bind, that is, to the N7H side first and then to the N9H side depends on assignment of the order of the N7H and N9H stretches. Previous experimental assignment of the order of these stretch vibrations, based on methyl substitutions, conflicted with that predicted by calculation.[12] We note that the hydrogen bond formed by a water to the N7H(a) and the C═O(a) should be stronger than a bond formed with the N9H(b) and N3(b), which is consistent with our assignment of which water binds first.

The primary conclusion from these data is that a single H_2O suffices to stabilize one specific base pair structure, relative to one that in the absence of solvent is close in energy. In earlier work we did not observe the GG structure that is predicted to be the lowest in energy.[11] We speculated that this might be due to its symmetry, causing exciplex formation that might result in a large spectral shift. If this were the case one might expect addition of a water molecule to break the symmetry; however, we do not appear to observe this structure here either. One possible explanation might be a short S1 state lifetime for this specific structure, analogous to the selective photochemistry we found for the Watson−Crick structure for guanine−cytosine clusters.[13] Although in our initial experiments we do not observe stacking for the GG base pair upon addition of two water molecules only 20% of the structures are predicted to stack, and this population could be below our detection limit. Specific substitutions will change, and usually increase, the ratio of stacked-to-hydrogen-bonded structures.[14] In ongoing experiments, we are investigating possible stacking and other structural changes in these and other base pairs caused by the addition of an increasing number of water molecules.

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