REMPI Spectroscopy of Laser Desorbed Guanosines

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To observe fundamental properties of DNA building blocks it is desirable to study individual nucleosides in the gas phase without interference from solvent molecules, or macromolecular structure. As a first step, we have recently reported the first vibronic spectrum of the nucleobase guanine, obtained by a combination of laser desorption, jet cooling, and resonance enhanced multiphoton ionization (REMPI).[1] Although guanine is important as a chromophore in DNA, it is more realistic for understanding the photochemistry of DNA to study the nucleosides. Those are even harder to vaporize intact because they are thermally more labile and, with their larger molecular weights, have still lower vapor pressures. Using laser desorption, we have now succeeded in forming a molecular beam of nucleosides, and we report the first REMPI spectra of a series of individual guanosines, namely guanosine (Gs), 2′deoxyguanosine (2′deoxyGs), and 3′deoxyguanosine (3′deoxyGs). We compare our results with computations at the HF 6-31G(d,p) level. The results suggest the occurrence of two different conformations, each probably stabilized by internal hydrogen bonds. One of those two conformations is absent in 2′deoxyGs implying that the 2′ hydroxyl group is required for its stabilization.

Spectroscopic properties of guanosines have been studied primarily by Raman techniques in solution.[2−9] A great deal of attention has been given to potential Raman markers for hydrogen bonding and for structural conformation. Observation of hydrogen bonding by Raman spectroscopy requires identification of vibrations that depend strongly on those specific atoms in guanine, that serve as either proton donor or acceptor. However, most vibrations involve the concerted motion of multiple atoms, and therefore correlation of marker frequencies with specific hydrogen bonding sites is not straightforward. Guanosine vibrations involving motion along the glycosidic bond may provide conformational markers if their frequencies are sensitive to puckering of the ribose ring or for rotation around the sugar−base bond. Interpretation of these markers requires careful analysis of complex vibrational modes. On the other hand, different conformations can be observed much more directly by vibronic spectroscopy when they produce multiple origins. As we will show below, we observe two origins in our spectra, which we can associate with the *syn* and the *anti* orientations of the base relative to the ribose moiety.

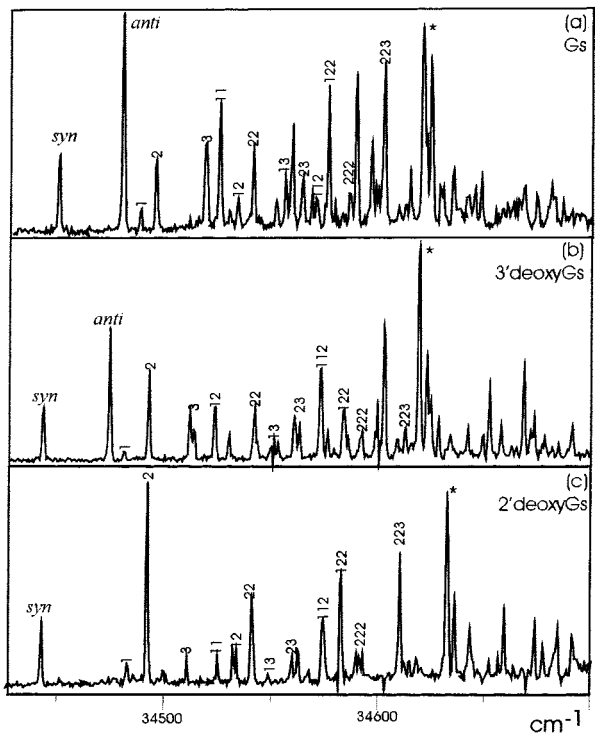

Figure 1. REMPI spectra of (a) guanosine, (b) 3′deoxyGs, and (c) 2′deoxyGs. In this energy range guanine itself does not exhibit any vibronic activity since its lowest energy peak is at 238 cm^{-1} above the origin. The *syn* and *anti* labels indicate origins of two possible conformers, and the numbers indicate vibrational modes and their combinations and overtones, for example 122 indicates one quantum of mode 1 and two quanta of mode 2; f indicates fundamental vibration.

We have published details of our setup for laser desorption jet cooling REMPI spectrometry elsewhere.[10] Sample preparation consisted of depositing neat material in powder form on graphite substrates. We moved the substrate slowly while acquiring spectra, gradually exposing fresh material. For desorption we used pulses from a Nd:YAG laser at 1064 nm with fluences on the order of 1 mJ/cm^2. Desorbed neutral molecules were entrained in a supersonic expansion with Ar drive gas, injected by a pulsed solenoid valve. Downstream, the entrained molecules were one-color two-photon photoionized, and the ions were detected in a reflectron time-of-flight mass spectrometer. The first photon resonantly excites the molecule, while a second photon from the same laser ionizes the excited molecule. By varying the wavelength while monitoring specific mass peaks we obtained mass selected excitation spectra. The typical ionization laser fluence was on the order of 0.1 mJ/cm^2.

Figure 1 shows the REMPI spectra of (a) Gs, (b) 3′deoxyGs, and (c) 2′deoxyGs. We assign the lowest-energy peak in each of the spectra as a 0−0 transition to the S_1 excited state. Careful scans to lower energy by 1000 cm^{-1} do not reveal any additional peaks. The same was true when performing two-color ionization with a second photon at 193 nm. Therefore, we do not believe that we are observing a cutoff in the spectrum related to the ionization potential of Gs. Furthermore we have measured the ionization potential of guanine as 8.1 eV by two-color ionization.[11,12] That is 1 eV less than the two-photon energy at the Gs origin.

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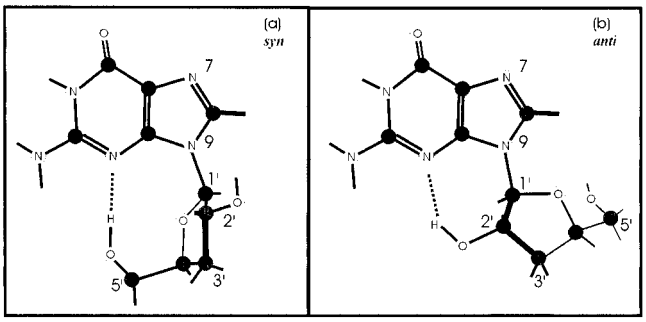

Figure 2. *Syn* (a) and *anti* (b) conformations of 3′deoxyGs. Gs has −OH in both 2′ and 3′ positions. 2′deoxyGs has −OH only in the 3′ position. Dotted lines indicate hydrogen bonding.

The origin of Gs is blue-shifted 1565 cm^{-1} with respect to that of guanine. This shift is of the same order of magnitude as the shifts which we have found for other substituents at the same nitrogen. The origins of 9-methyl guanine, 9-ethyl guanine, and 9-butyl guanine are blue-shifted with respect to guanine by 1730, 1685, and 1630 cm^{-1} respectively.[12] We assume that the spectral shift upon substitution at the sugar position is due to changes in the electron density distribution in the S_1 state. The spectral range is at the edge of that of sunlight at the earth surface. Therefore we note that the blue-shift of guanosine with respect to guanine may decrease the sensitivity of the DNA's major chromophore to photochemistry.

Figure 2 shows two molecular structures of 3′deoxyGs which we obtained by minimizing the energy. All calculations were performed at the Hartree−Fock level (6-31G(d,p) basis set) using the program GAUSSIAN 98. All structures have been fully optimized with 8−10 hartree as SCF convergence criterion and 1.5×10^{-5} Hartree/bohr and Hartree/degree, respectively, as convergence criterion for the gradient optimization of the structures. Both structures exhibit intramolecular hydrogen bonds. The *syn* conformation (panel a) is stabilized by hydrogen bonding between the guanine N3 and the 5′OH on the sugar. The *anti* conformation (panel b) is stabilized by hydrogen bonding between the guanine N3 and the 2′OH on the sugar. The energy difference between the two structures is 1.5 kcal/mol on the HF 6-31G(d,p) level including zero point energy correction. For 2′deoxyGs the difference between the *syn* and the *anti* conformation is twice as large because in that case the *anti* structure cannot be stabilized by hydrogen bonding. The concept of two restricted conformations of the base relative to the ribose was first proposed by Donohue and Trueblood.[15] There have been a number of conflicting studies as to which conformation is preferred under different conditions, in solution and in the crystalline form.[16,17] Studying Gs in the gas phase greatly simplifies this problem by providing spectroscopy of individual, unperturbed molecules.

The red-most origins for Gs and for 3′deoxyGs are at the same frequency, 34443 cm^{-1}, while the red-most origin for 2′deoxyGs is shifted by only 7 cm^{-1} relative to the other two. Therefore, we assume that those are origins of similar conformations, which are not strongly influenced by the presence or absence of the 2′ and 3′ hydroxyl groups on the sugar. The spectra are dominated by peaks in the energy range below 200 cm^{-1}, where the guanine spectrum is very sparse since the first peak that we observed in the guanine spectrum is at 238 cm^{-1} above the origin.[1] We attribute these low-frequency guanosine peaks to vibrations of the sugar moiety with respect to the base, involving the N9−C1′ bond. The three lowest frequencies in each spectrum are in reasonable agreement with calculated frequencies, given in Table 1, obtained by performing a normal-mode analysis on the optimized geometries using analytical gradients of the energy. Since the calculated frequencies are for the ground state, while the measured frequencies are for the excited state, the reasonable agreement suggests the absence of large geometry changes. These frequencies correspond to three different motions of the sugar moiety with respect to the base The lowest energy mode is a torsion; the next two are a wagging motion in the plane of the base and perpendicular to it, respectively. We find that we can explain almost all of the peaks up to 170 cm^{-1} in the 2′deoxyGs spectrum by combinations and overtones of just those three frequencies, as indicated in Figure 1. We can also assign equivalent peaks in the spectra of Gs and of 3′deoxyGs. However in those two spectra there appears an additional set of peaks. Those additional peaks are likely to be due to a second conformation, the absence of which in 2′DeoxyGs implies that it is a structure involving the 2′OH group. This suggests that this must be the *anti* conformation with 2′OH−N3 hydrogen bonding. In this interpretation the red-most of this second set of peaks is the origin of the *anti* conformation. We have labeled the proposed origins of the two conformations as *syn* and *anti* in Figure 1. We have not attempted to further interpret this second set of peaks because we cannot do so consistently and we do not seem to observe the low-frequency fundamental vibrations. As a result, assignment of overtones and combination bands would be too arbitrary. The peak in each spectrum marked with an asterisk does not fit in the vibrational scheme for the *syn* conformations. These peaks are relatively intense, and unlike peaks for the *anti* conformation, it also occurs in 2′deoxyGs but at a higher frequency than in the other two molecules. This can mean one of several things. This may be another vibration of the *syn* conformation, the frequency of which is affected by the absence of the 2′OH. It is also possible that this is another conformation or tautomer in the guanine moiety. We are planning spectral hole burning experiments in order to clear up this issue and to obtain vibrational spectra of the individual conformers.

Table 1. Lowest Three Observed REMPI Frequencies and Calculated (calc) Ground State Vibrational Frequencies in cm^{-1} for Three Guanosines

mode	2′deoxyGs	calc	Gs	calc	3′deoxyGs	calc
1	40	43	38	40	38	40
2	49	55	45	51	50	54
3	68	79	69	80	69	89

In summary, we have obtained the first REMPI spectra of individual guanosines, put in the gas phase by laser desorption/jet cooling. The spectra can be interpreted as resulting from two conformers, *syn* and *anti*, each stabilized by intramolecular hydrogen bonding.

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