REMPI Spectroscopy of Jet-Cooled Guanine

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There have been many theoretical studies of vibrational and electronic states of DNA bases.[1−3] These are complicated by multiple lone pair electrons, limited symmetry, and possible tautomerism. Experimentally there have been many reports of absorption spectroscopy,[4,5] IR data,[6,7] and Raman spectroscopy.[8] Samples have been in the form of vapor, solutions, polycrystalline material, single crystals, and cold matrices. Gas-phase studies can offer the advantage of eliminating intermolecular interactions. However, if gas-phase spectroscopy is to provide vibrational resolution, it requires entrainment of the molecules in a supersonic expansion in order to reduce their internal temperature. Brady et al. studied the pyrimidine bases, uracil and thymine, in a supersonic jet and obtained only broad spectra.[9] Brown et al. have reported microwave spectra of all bases except guanine and assigned tautomers.[10−13] Kim et al. have formed clusters of nucleotides and water molecules in a jet and measured their ionization potentials.[14] Caminati et al. have recorded the millimeter wave spectrum of jet-cooled purine.[15] Viant et al.[16] have used a slit nozzle to obtain high-resolution IR spectra of jet-cooled uracil. While the latter work reveals details of the molecular ground state, we employ REMPI spectroscopy of jet-cooled DNA base molecules to probe the first excited electronic state. After exploring the basic building blocks we aim to extend these studies to oligonucleotides and to their hydrogen-bonded clusters. Since those cannot be vaporized intact by heating, we have based our approach on laser desorption.

We have published details of our setup for laser desorption jet cooling REMPI spectrometry elsewhere.[17] Sample preparation consisted of depositing material in thin layers on graphite substrates. For desorption we used pulses from a Nd/YAG laser at 1064 nm with fluences of the order of 1 mJ/cm^2. Desorbed material was entrained in a supersonic expansion with Ar drive gas, injected by a pulsed solenoid valve. Desorption took place on the vacuum side of the valve, within 2 mm of the 1 mm opening to obtain optimum cooling of the internal degrees of freedom. We occasionally employed other drive gases to double check for the absence of cluster formation. A signal measured at the parent mass could be due to dissociating van der Waals clusters. In that case the wavelength would be shifted due to the van der Waals interaction. Clustering with different gases would then lead to different spectral shifts. Obtaining peaks at the same wavelength for different drive gases is therefore an indication that the spectra were not modified by cluster shifts. Downstream, the entrained molecules were two-photon photoionized and the ions were detected in a reflectron time-of-flight mass spectrometer. The typical fluence was of the order of 1 mJ/cm^2. The second photon came either from the same laser (one-color REMPI) or from another one (two-color REMPI). For the latter approach we employed an excimer laser at 193 nm, with a variable time delay with respect to the first laser. By varying the wavelength of the first photon while monitoring specific mass peaks we obtained mass selected excitation spectra.

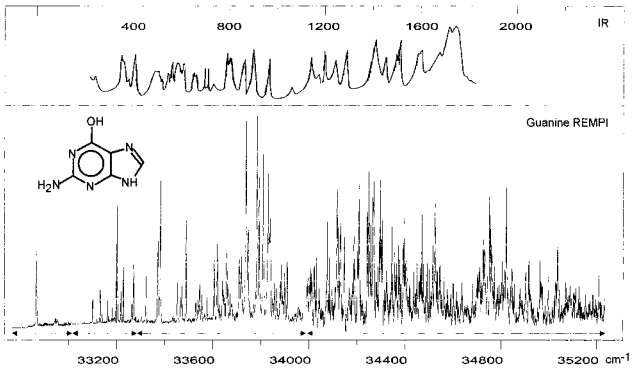

Figure 1. REMPI spectrum of jet-cooled guanine. The spectrum consists of four parts, as indicated by the horizontal arrows, that were recorded independently. In each part the ionization laser fluence varies such that it is highest in the center of the respective range. The peaks are not normalized for laser fluence. The top panel shows IR data from ref 8, plotted relative to the lowest energy peak in the REMPI spectrum for comparison.

Static gas-phase absorption, recorded in a heated cell, shows a structured band with a maximum at 293 nm and an onset well beyond 300 nm.[5] Absorption as well as fluorescence excitation of guanine in a nitrogen matrix at 15 K also shows several bands with an onset for the first one between 300 and 310 nm.[18] Figure 1 shows the REMPI spectrum of jet-cooled guanine. The figure shown is a compilation of three spectra, recorded at adjacent wavelength ranges, as indicated by the horizontal arrows. The peak heights are not normalized for laser fluence, which varied within each range. The lowest energy segment is shown with significantly reduced intensity. We recorded all spectra at the parent mass of 151 Da. The mass spectra contained fragments as well, mainly at masses 134, 135, 109, and 110 Da. Although we did not study the wavelength dependence at the fragment masses in detail, it was essentially identical to that at the parent mass. This indicates that insofar as fragmentation takes place, it does so after ionization and not during desorption.

The lowest energy peak that we observed was at 32 878 cm^{-1}. We carefully scanned further to the red of this peak with two-color ionization up to 32 150 cm^{-1} without finding any additional peaks. We may therefore tentatively assign this peak as the 0−0 transition. At this point we merely compare our results with published IR data on polycrystalline samples,[8] samples in cold

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matrices,[6] and normal mode calculations.[6,7,19] In Figure 1 we have plotted the polycrystalline IR data relative to the base peak at 32 878 cm^{-1}. We note that all calculations cited here are in fair agreement with these and other published IR data. Our data represent vibronic transitions to the electronically excited state while all other data probe the molecular ground state. Therefore the comparison is relevant only if there is no significant geometry change between the two states. One can recognize similarities in global features if one would assume that the vibrational spacings in the excited state appear to be about 10% larger than those in the ground state. This would suggest a slightly more attractive excited state. We note that Stepanek et al. reported agreement between ground- and excited-state vibrational levels of adenine, based on a vibronic absorption contour measured at 10 K in the solid phase.[20] They assign excited-state vibrations to levels at 216, 336, 486, 515, 579, 678, 854, and 1485 cm^{-1} above the origin. Following the normal mode calculations, which are used to interpret the IR data, one expects ring breathing vibrations in guanine around 900 cm^{-1}. Since these may be expected to exhibit significant intensity in REMPI spectra, they are likely to correspond to the group of peaks around 33 800 cm^{-1} in our spectrum. Another set of ring vibrations shows up in the IR data at about 1700 cm^{-1}. This likely corresponds to the REMPI features around 34 700 cm^{-1}, implying a small shift to higher energy. A detailed analysis of the spectrum should allow more precise assignments and will be published later.

Guanine is known to usually exist in solution in a keto rather than in the enol form. According to calculations, the ketone is also the more stable tautomer by 4.95 kcal/mol.[21,22] This is important because the ketone conformation is required for hydrogen bonding in Watson−Crick pair formation and therefore it is the form in which it occurs in DNA. In the polycrystalline sample guanine is also predominantly in a ketone form, as can be determined from the C═O stretch region in the IR spectrum. On the other hand, in the matrix there appear also enol tautomers, and the result is a much larger number of lines. We cannot measure the C═O stretch region in the present experiment because it is at too short a wavelength. However, it is possible that the lowest energy tautomer is frozen predominantly in our jet expansion. From a detailed analysis of the spectrum as well as additional measurements at shorter wavelength and spectral hole burning experiments we may be able to determine whether multiple tautomers are present in the jet-cooled conditions. If they are absent, this could constitute an experimental confirmation of the notion that this tautomeric form is indeed the more stable one.

The ionization potential of guanine has been reported as 8.24 eV (66 456 cm^{-1}).[23] This implies that at wavelengths greater than 300.9 nm (less than 33 228 cm^{-1}), two photons of the same energy would be insufficient to ionize the molecule. To probe the region close to the probable ionization limit we have recorded spectra from 32150 to 33330 cm^{-1} with both one and two lasers. The fluence from the second laser, at 193 nm, was adjusted such that it alone did not produce any ions. Over the entire region the second laser produced an enhancement; however, we did not record any peaks with two-color ionization that could not be obtained with one-color ionization as well. This is also true for peaks at energies less than 33228 cm^{-1}. This may put into question the literature value for the ionization potential. However, we note that at the electric field of 2000 V/cm, which we employ for extraction, the effective ionization potential may well be lowered by at least 150 cm^{-1}.[24] The remaining discrepancy, of the order of 0.06 eV, may well be within the margin of error by which the ionization potential could be determined. Furthermore, the quoted ionization potential is the vertical ionization potential as measured by photoelectron spectroscopy while the adiabatic ionization potential is 7.7 eV. The latter number may well be relevant for sequential two-photon ionization in which the molecule can have time to relax in the intermediate state before absorbing the second photon. Finally, it is possible that, following excitation to a high Rydberg state, a third photon is absorbed.

Employing two-color ionization with a variable delay between the lasers allows us also to address the lifetime of the excited state. DNA bases in solution and at room temperature are reported to have a very short-lived first excited singlet state, which undergoes rapid internal conversion with a lifetime of the order of picoseconds and corresponding quantum yields of the order of 10^{-4}.[25,26] It has been suggested that evolution has arrived at molecules for genetic material with a minimized propensity for photochemistry. On the other hand, Longworth et al. have observed phosphorescence with decay times of the order of a second from purines and nucleotides at 77 K,[27,28] while Polewski et al. measured a fluorescence lifetime of 10 ns for matrix-isolated guanine.[18] This suggests that internal conversion is not the dominant decay channel at low temperature and in the absence of solvent interactions which can take up excess energy. This should then also be the case under our jet-cooled conditions. If the lifetime in the gas phase were of the order of picoseconds, it is doubtful that we would be able to observe multiphoton ionization at all. More importantly, we can readily observe ionization with a second laser, 193 nm, which is fired several microseconds after the first. The second laser alone does not produce any ions. Therefore we can be certain that it ionizes molecules from a long-lived excited state, which results from the resonant excitation by the first laser. The most probable explanation is that under jet-cooled conditions there is intersystem crossing to the same triplet state that is responsible for the observed phosphorescence. From the lowest triplet state at 27 778 cm^{-1} [27] a single 51813 cm^{-1} photon from the second laser suffices to ionize the molecule. In the present setup the excited molecules leave the source region in 5−10 μs. This imposes a limited window on the lifetimes that we can measure by varying the laser delays. At this point we can only state that indeed the lifetime exceeds our measurable range.

We have presented the first vibronic spectroscopy to our knowledge of guanine in the gas phase. We have obtained an excited state spectrum of the nucleotide by REMPI, following laser desorption and jet cooling. The spectrum of this purine-based nucleotide shows a sharp structure over a long wavelength range. This is in marked distinction from the spectra of the pyrimidines, which we have also reproduced in our laboratory. We have tentatively assigned the S_{0-0} at 32 878 cm^{-1}. With this assignment we can compare this spectrum with existing IR spectra of guanine in the solid phase and with theoretical calculations. The excited state shows a lifetime exceeding 10 μs, as measured with two-color ionization.

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