Vacuum-ultraviolet ionization spectroscopy of the jet-cooled RNA-base uracil

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Adiabatic ionization potential (9.3411 ± 0.0008 eV) and cationic vibrational structure of the jet-cooled RNA-base uracil are both accurately and precisely determined for the first time using a vacuum-ultraviolet mass-analyzed threshold ionization spectroscopy.

In damage and mutation of genes by radiation, the ionization of nucleic acid bases acts as an initiation step in the charge hopping process occurring along nucleic acid strands.[1–3] Therefore, for the molecular-level understanding of the nucleic acid damage/mutation, one of the fundamental physical properties that is most crucial would be the relative highest-occupied molecular orbital (HOMO) energy levels of individual nucleic acid bases. Even though a number of experimental and theoretical studies on this subject have been carried out in recent decades,[4–11] experimental study on the ionization of nucleic acid bases still lacks reliable and quantitative measurements. Photoelectron spectroscopy (PES) of nucleic acid bases has provided only crude values for the ionization potentials of bases with large uncertainties. Consequently, the previously reported values for the ionization potential of an RNA base uracil, for example, are scattered in a wide energy range.[4–8] Furthermore, because of an intrinsic poor energy resolution in PES, no vibrational structure of base cations has been reported to date. Even though there exist many experimental difficulties such as that nucleic acid bases have very low vapor pressures and are easily decomposable, one should apply the currently available state-of-the-art experimental technique to obtain more exact information about these important biological building blocks.[12]

One of the main differences between RNA and DNA is the fact that RNA has the uracil base, instead of the thymine base in DNA. The methylation of uracil gives thymine, and this simple chemical difference between uracil and thymine results in big differences not only in the chemical reactivity but also in the physical properties of RNA or DNA, which are critical in biological processes. We wish to provide the fundamental information which can be used for the clarification of the latter in this report. Here, we employ a vacuum-ultraviolet mass-analyzed threshold ionization (VUV-MATI) spectroscopy for the determination of the accurate ionization potential and cationic vibrational structure of the RNA base uracil. VUV-MATI spectroscopy has some advantages compared to the more conventional (1+1′) two-photon MATI spectroscopy[12,13] in terms

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of its universal applicability to any molecular system and the fact that the geometrical change upon ionization can be directly inferred from the VUV-MATI spectrum. The VUV laser pulse (131–133 nm) was generated using a four-wave mixing process in a Kr cell (2 Torr) using the two-photon Kr $5p[1/2]_0$–$4p^6$ transition at 212.550 nm and tunable visible laser pulse output in the 531–560 nm range. The VUV laser output ($\Delta t \sim 5$ ns) was then separated from the UV and visible fundamentals by the edge of a collimating CaF_2 lens to be overlapped with the molecular beam of uracil seeded in Ar carrier gas. The supersonic jet containing the mixture of uracil and Ar was formed from a high-temperature nozzle (modified General valve, 0.5 mm diameter orifice) heated to 280–300 °C. The long-lived high-lying Rydberg states of uracil, directly reached by the VUV photon, are pulsed-field ionized after a delay time of 25 microseconds following the VUV laser pulse to give the MATI spectra in Fig. 1. No spoil field was necessary, since the long delay time between the VUV laser pulse and the pulsed ionizing electric field ensured the separation of MATI ions from direct ions. It should be noted that we used a high-voltage pulsed field (430 V/cm) for a low-resolution MATI spectrum in Fig. 1(a), while a low-voltage pulsed field of 8 V/cm was used for a highly-resolved MATI spectrum in Fig. 1(b). The origin band is strongly observed at the VUV photon energy of 75 341 cm^{-1}, corresponding to the adiabatic ionization energy of uracil at 5 K. Since the MATI peak position strongly depends on the pulsed electric field strength, the true ionization potential is deduced from the extrapolation of MATI peak positions plotted as a function of the square root of the pulsed electric field. After such a correction,[12] the accurate ionization potential of uracil is
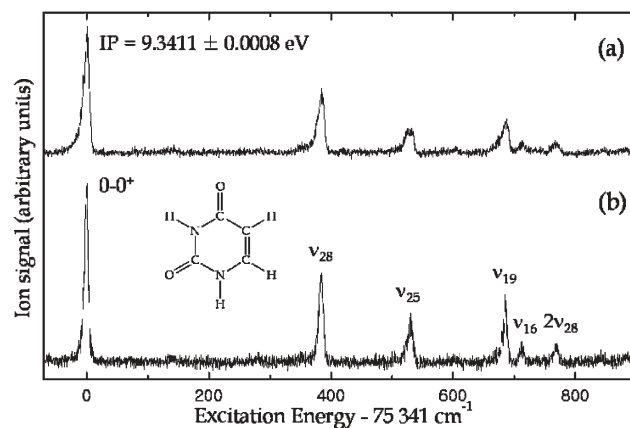

Fig. 1 VUV-MATI spectrum of the jet-cooled uracil at the (a) low-resolution and (b) high-resolution modes. See Table 2 for the normal mode description of the observed vibrational bands.

Table 1 Experimental ionization potentials of uracil

IP (eV)	Method	[Ref.]/year
9.3411 ± 0.0008	VUV-MATI	This work
9.15 ± 0.03	Photoionization mass spectrometry (PIMS)	[8]/2005
9.59 ± 0.08	Electron ionization	[7]/2004
9.53	Photoelectron spectroscopy (PES)	[6]/1996
9.32 ± 0.05	PIMS	[5]/1976
9.50 ± 0.03	PES	[4]/1975
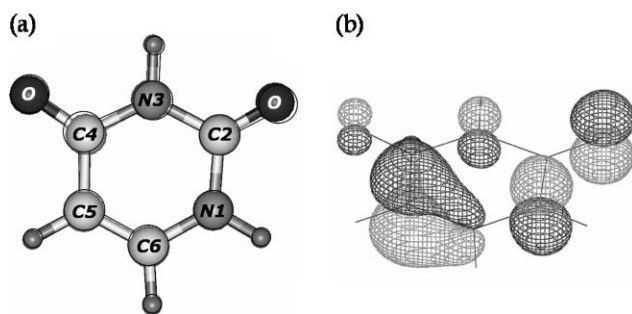
Fig. 2 Minimum energy structures of uracil in (a) the neutral ground state (circle and rod) and the cationic ground state (ball and stick). (b) The highest occupied molecular orbital diagram of π_3, calculated by the density functional theory using the (U)B3LYP method with the 6-311+G(d,p) basis set, is sketched on the structure of the ground cationic uracil.

Table 2 Experimental and *ab initio* calculated vibrational frequencies (in cm^{-1}) of the uracil cation

Obs.	Calc.		Assign.	Normal mode description[c]
	DFT^a	$MP2^b$		
0			$0-0^+$	
384	342	386	ν_{28}	C=O bend. (sym.)
530	522	525	ν_{25}	antisym. ring def.
686	774	701	ν_{19}	ring breathing
713	992	894	ν_{16}	ring def. + C4–C5 str. + C4–N3 str.
768			$2\nu_{28}$	

[a] DFT (UB3LYP/6-311+G(d,p)) not-scaled vibrational frequencies calculated for the optimized ground state of the uracil cation. [b] MP2 (UMP2/6-311+G(d,p)) not-scaled vibrational frequencies calculated for the optimized ground state of the uracil cation. [c] Normal mode descriptions are based on the inspection of nuclear displacement vectors of each mode (Fig. 2). The normal mode is numbered from the highest frequency in descending order. sym.: symmetric, antisym.: antisymmetric, str.: stretching, def.: deformation, bend.: bending.

determined to be 9.3411 ± 0.0008 eV. The ionization potential of uracil determined in this work is compared with previously reported experimental values, Table 1.

The VUV-MATI spectrum in Fig. 1 provides the vibrational structure of the uracil cation. The pattern of the vibrational spectrum reflects the shape of the HOMO from which an electron is ejected in the first ionization according to the Franck–Condon principle. *Ab initio* calculations (DFT, UB3LYP/6-311+G(d,p))[14] predict that the cation ring structure is asymmetrically distorted on the molecular plane, as depicted in Fig. 2. Thus, in-plane ring distortion modes are expected to be optically active in the VUV-MATI spectrum. Vibronic bands with low vibrational frequencies

of 384, 530, 686, and 713 cm^{-1} are thus assigned as in-plane ring distortion modes of the uracil cation using the results of *ab initio* calculations.[14] Two different computational methods (MP2, DFT) give different vibrational frequencies for the same normal mode, and these are listed in Table 2 to be compared with experiment. The structural change of uracil upon ionization is not dramatic, but it is quite clear that there is a significant asymmetric in-plane ring distortion as the uracil base is ionized, as indicated in Figs. 1 and 2. This structural change in the RNA base ionization is quite important and should be taken into account when explaining the charge hopping process at the molecular level.

In this communication, we have presented the highly-resolved ionization spectrum of the RNA base uracil for the first time. The adiabatic ionization potential of the jet-cooled uracil is both accurately and precisely determined to be 9.3411 eV. The uracil cation, in its ground state, is found to be asymmetrically distorted on the molecular plane. The VUV-MATI spectrum gives vibrational frequencies of the uracil cation for some in-plane ring distortion modes, and they are compared with *ab initio* frequencies. Our first ionization spectroscopic work on the RNA base uracil will be extended to the spectroscopy of other nucleic acid bases as well as associated clusters. The accurate and precise physical properties of biological building blocks such as those reported here will stimulate more rigorous theoretical calculations in this important field.

Notes and references

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