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Threshold behavior in metastable dissociation of multi-photon ionized thymine and uracil

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Abstract

Microsecond-timescale HNCO loss has been observed from single-color multi-photon ionized pyrimidine nucleobases in the gas phase. Photon energy thresholds for the metastable channels have been measured at 5.55±0.02 eV for thymine and 5.57±0.02 eV for uracil. We argue that these results can be attributed to accessing the molecules' S_1 states with additional vibrational energy matching the threshold energy for HNCO loss from the radical cation. Combined with previous photoionization energies, this enables the S_1 adiabatic energies to be deduced: 3.67±0.07 eV for thymine and 3.77±0.07 eV for uracil. These values are consistent with recent calculations.

Keywords

Thymine; uracil; pyrimidine nucleobases, metastable fragmentation; multi-photon ionization; mass spectrometry

Graphical abstract
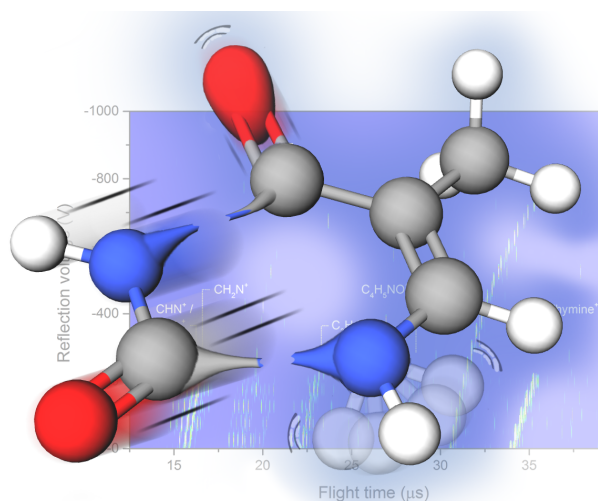
1. Introduction

The neutral electronic excited states and ionic states of DNA and RNA bases have been studied extensively in recent years[1,2] with the particular aim of understanding the response of these biological building blocks to UV irradiation. Dynamical calculations[3] and time-resolved spectroscopic measurements on isolated pyrimidine nucleobases[4] have revealed a great deal of information on the relaxation pathways from their optically bright $S_2(\pi\pi^*)$ states. Internal conversion to the electronic ground state (either directly or via S_1 states of mainly $n\pi^*$ character) dominates $S_2(\pi\pi^*)$ deactivation[5,6], although intersystem crossing to long-lived triplet states has also been reported[7,8]. The majority of previous experiments have probed the relaxation dynamics of nucleobases following access to low vibrational levels of S_2, for example pumping with the third harmonic of a Ti-sapphire laser (4.65 eV compared with the calculated S_2 origin of thymine at 4.49 eV[9]). In the present multiphoton ionization (MPI) experiments, isolated thymine and uracil are excited at photon energies of 5.39-5.64 eV, where significant vibrational excitation increases the range of possible relaxation pathways. In particular, tautomeric transitions and ring opening conical intersections have been predicted theoretically[10,11]. Our recent studies of uracil indicate that the analysis of fragment ion production from multiphoton ionized molecules can provide evidence for structural changes in neutral excited states[12,13]. Matsika and co-workers[14–16] have also studied fragment ion production from multiphoton ionized nucleobases and argued that the detection of intact singly ionized uracil (uracil$^+$) in 260 nm pump - 780 nm probe experiments could only be traced to ionization from S_2, whereas the $C_3H_3NO^+$ yield (uracil$^+$ less HNCO) could provide information about the wavepacket evolution on both S_2 and S_1. This paper reports metastable dissociation of multiphoton ionized nucleobases for the first time and considers its potential to provide new insights into the valence excited state energies and dynamics of these important biomolecules.

2. Experimental

The experimental system has been described in previous publications[12,13,17,18]. Briefly, helium carrier gas (0.6-0.8 bar) seeded with sublimated thymine or uracil (both from Sigma-Aldrich with stated purity ≥99%) flowed continuously through a 50 μm diameter pinhole into a pumped chamber (500 ls^{-1}) to form a supersonic jet. The powder temperature (250 °C) was comparable with or lower than those applied in previous mass-spectrometry and IR spectroscopy experiments that reported no evidence for thermally driven decomposition, isomerization, tautomerization, or reactivity in uracil or thymine following sublimation in an inert gas[19]. The carrier gas, the driving pressure, and the pumping speed in the expansion chamber were chosen to minimize clustering. The jet passed through a skimmer into a second pumped chamber and crossed an Nd:YAG pumped and frequency doubled dye laser beam (*Continuum Powerlight II 8000 - Sirah Cobra-Stretch*, repetition rate 10 Hz, pulse width 7 ns, pulse energy 100-2,000 μJ, wavelength 219-277 nm). The resulting ions were detected using a reflectron time-of-flight (TOF) mass spectrometer (supplied by KORE Technology) shown schematically in Fig. 1 with the *field free region* (FFR) held at a voltage of V_{FFR}= -2,027±1 V. The voltage on the reflection electrode could be adjusted to test for metastable dissociation, as described in section 3. The pre-amplified ion signals were timed using a *Fast Comtec P7887* time-to-digital conversion (TDC) card.

The data acquisition system was based on a *LabView* application interfacing with the TDC card and a laser pulse energy meter (*Spectrum Detector SPJ-D-8*). A convex lens on a slider was used to control the diameter of the laser beam when it crosses the molecular beam and the average laser pulse energy was adjusted using the delay between the pulses triggering the xenon flash lamps and the *Q-switch* of the Nd:YAG laser.

3.   Results and Discussion

Fig. 2 shows MPI TOF mass spectra of thymine and uracil as a function of the reflection voltage, V_R. The mass spectra have not been calibrated in the figure because the proportionality of flight time against $\sqrt{\frac{m}{z}}$ breaks down for delayed fragmentation. Each band falls to zero at a characteristic reflection voltage, $V_R(0)$. The difference between $|V_{FFR}|$ and $|V_R(0)|$ gives the ion's kinetic energy (KE) in eV immediately prior to entering the reflectron optics. For thymine[+] and uracil[+], $V_R(0)$= -212±1 V. This corresponds to the voltage at the point in space where the focused laser beam crossed the molecular beam (explained in detail by Ryszka et al.[13]). Hence the KE of the radical cations in the field free region (FFR) was 1815±2 eV. For fragment ions produced *promptly*[*] after ionization, $V_R(0)$ is slightly more negative due to dissociative KE release (typically up to several eV for single ion production by MPI[20]) but fluctuations of ±1 V on the mass spectrometer voltages prevented a detailed analysis of this effect.

The *metastable bands* extending to more negative V_R values in Fig. 2 are due to the fragmentation of ions during their first journey through the FFR (1.3-15.7 µs after the laser pulse). The resultant ions have relatively low kinetic energies and so can be reflected by a weaker field. $V_R(0)$= -830±1 and -907±1 V for the metastable bands in Fig. 2a and b, respectively. Hence the KE of the relevant ions immediately before entering the reflectron optics were 1197±2 and 1120±2 eV, corresponding to 65.9±0.2% and 61.7±0.2% of the KE prior to fragmentation. Comparisons between the calculated and measured flight times ruled out metastable dissociation of a fragment ion. This defines the metastable fragment ion m/z values as 83.1±0.3 from thymine[+] and 69.1±0.3 from uracil[+]. Based on thermochemical[21] and DFT[22] calculations, the m/z 83 and 69 ions can be assigned to $C_4H_5NO^+$ and $C_3H_3NO^+$ following HNCO loss from the respective radical cations. HNCO loss is widely recognized as the first step in the main sequential fragmentation pathways of both thymine[+] and uracil[+]. The ions at m/z 83 and m/z 69 are also notable for having the lowest appearance energies of any fragment ions from thymine and uracil radical cations[21,23].

Rice et al.[24] carried out electron impact ionization (EII) studies on a range of pyrimidine derivatives and were able to recognize metastable fragmentation processes using a double focusing mass spectrometer. Although they did not show their data in detail (for example, no timeframes were mentioned), they reported delayed HNCO loss from uracil[+] and stated that the fragmentation of

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[*] The present experiments were only sensitive to delays ≥100 ns between ionization and dissociation. Hence, we have chosen to describe more rapid dissociation as *prompt*.

thymine[+] was "perfectly analogous" to uracil[+] with respect to the first step of the main sequential fragmentation pathways. Our observation of metastable HNCO loss from multi-photon ionized thymine[+] and uracil[+] is therefore broadly consistent with the EII literature. However Rice et al.[24] did not discuss the mechanism for metastable HNCO loss in contrast with prompt dissociative ionization. The photon energy and fluence dependence of these channels in MPI measurements can provide insights that are not available from EII experiments. We have previously observed metastable HNCO loss from multi-photon ionized uracil and demonstrated that this was a 2-photon process at 220 nm[12] but were unable to offer a mechanistic interpretation.

Fig. 3 shows the metastable HNCO loss signal from thymine at 220 nm (5.64 eV) as a function of the laser pulse energy. The photon order (α) can be estimated from the ion counts per pulse (I) and the pulse energy (E) using the perturbation theory expression $I=cE^{\alpha}$, where c is a constant[25]. To within the experimental uncertainty limits, the photon order for the metastable pathway (1.4±0.2) matched the photon order for thymine[+] production (1.7+0.3) in the same measurements (not plotted here). The fact that the photon orders are less than 2 indicates some level of saturation. The key conclusion is that the same number of photons was required for both processes, while thymine[+] production can reasonably be assumed to be a 2-photon process.

Fig. 4 shows the dependence of the metastable HNCO loss channels on the laser photon energy, at the same average fluence of 9×10^{7} Wcm^{-2}. Thresholds are visible at 5.55±0.02 and 5.57±0.02 eV from thymine and uracil, respectively (notwithstanding reduced signal-to-noise ratios in the uracil data below 5.51 eV). These energies were determined as the midpoints between the highest energy at which the signal overlapped with the background level and lowest energy at which the signal was clearly higher than the background level. The uncertainty was then estimated as the energy spacing between the data points. The observation of a photon energy (i.e. wavelength) threshold for an MPI fragment ion is a strong indicator of a critical process in the neutral excited state dynamics[12]. Ionic state thresholds do not manifest themselves as photon energy thresholds because ionization can take place with various photon orders in the same measurement. If the total energy delivered by two photons is not sufficient to produce a given fragment ion, then the absorption of three or four photons can achieve this as long as the laser fluence is sufficiently high. To illustrate this crucial point, Fig. 5 compares MPI fragment ion production from thymine at 220 and 270 nm, at the same average fluence of 9×10^{7} Wcm^{-2}. Previous electron impact and single photon ionization studies of thymine revealed particularly strong signals at m/z 27, 28, 39, 54, and 55[21,24]. As expected for channels that can be accessed efficiently by direct excitation from the neutral electronic ground state[12], we see no evidence for threshold behavior in the MPI production of these fragment ions. The $C_3H_3^{+}$ (m/z 39) signal is worthy of particular attention as the single photon ionization appearance energy for this ion (14.4±0.1 eV[21]) is comfortably attainable with 3 photons at 220 nm (5.64 eV) but requires 4 photons at 270 nm (4.59 eV). This change in the required photon order does not manifest itself as a photon energy threshold. Accordingly, we are confident that the photon energy thresholds for metastable

HNCO loss shown in Fig. 4 are linked to critical processes in neutral excited states prior to the absorption of the ionizing photon.

The agreement of the photon energy thresholds for metastable HNCO loss from the two molecules (to within the experimental uncertainty of ±0.02 eV) provides a further indicator that that they cannot be understood solely in terms of ionic state energetics. The single-photon ionization energies of uracil are systematically higher than the equivalents of thymine[21]. In particular, the threshold energy for dissociative ionization with HNCO loss is 0.25±0.07 eV higher[21] from uracil than from thymine. In contrast with the differences in their ionization energies, the most recent calculated adiabatic energies of the lowest-lying singlet states of thymine and uracil agree to within ≤0.03 eV[26].

HNCO loss from thymine[+] and uracil[+] are understood to proceed via cleavage of the N_3-C_4 and N_1-C_2 bonds[21,22] (see Fig. 4). Arani et al.[22] calculated the potential energy barrier needed for HNCO loss from uracil[+] as 2.11 eV. Improta et al.[27] calculated the transition states of two reaction paths for HNCO loss from thymine[+] with potential energy barriers of 1.83 and 2.00 eV. The difference between the adiabatic ionization energy (AIE) of thymine and the appearance energy of $C_4H_5NO^+$ is (10.70±0.05 - 8.82±0.03 =) 1.88±0.06 eV[21]. This is very close to the corresponding value of (10.95±0.05 - 9.15±0.03 =) 1.80±0.06 eV for HNCO loss from uracil[+][21]. The similar energy requirement for HNCO loss from the two radical cations is not surprising as the HNCO group in question is relatively far from the methyl group that differentiates the two ions. The values given above come from Jochims et al.'s[21] photoionization experiments using synchrotron radiation and quadrupole mass spectrometry to measure *parent* and fragment ion appearance energies from thymine and uracil. Majdi et al.[28] have since reported the AIE of thymine as 8.913 ± 0.005 eV based on the analysis of vibrational structure in photoelectron spectra with supporting calculations. This is higher than Jochims et al.'s AIE of 8.82 ± 0.03 eV[21]. The difference between these results has previously been attributed to the temperature of the molecules prior to ionization: Jochims et al.[21] studied molecules from an effusive oven at 150-200ºC (3/2 KT ~0.06 eV) whereas Majdi et al.[28] probed jet-cooled thymine from a supersonic helium expansion. Despite the higher precision of Majdi et al.'s[28] AIE and the similarity of their experimental target with ours, Jochims et al.'s[21] results are more useful here because we require the differences between AIEs and fragment ion appearance energies from both uracil and thymine. As similar shifts to lower energy can be expected for AIEs and for fragment ion appearance energies, the differences between these values should not be modified significantly by the temperature of the molecule prior to ionization.

Our interpretation for the present threshold behavior is centered on the vibrational excitation of the nucleobase cation. If the total vibrational energy of the ion in its ground state (I_0) is at the threshold required for HNCO loss then a suitable redistribution of the activated modes can lead to delayed dissociation. The delay can be due to tunneling through a low potential barrier or a low rate constant for unimolecular fragmentation when the excess internal energy is small[29]. Electronic excitation of neutral thymine and uracil by single photon absorption in the present energy range results in

significant vibrational excitation. Due to the inefficient conversion of energy from vibrational modes to electronic modes, this vibrational energy *survives* the second photon absorption and results in an (at least) equivalently vibrationally hot ionic state. Competition with energy removal by the emitted electron means that the absorption of the ionizing photon is expected to be a far less efficient mechanism for vibrational excitation than the first photon absorption. Furthermore, the level of vibrational excitation in the jet-cooled molecules prior to photoexcitation can reasonably be assumed to be too low to affect the present threshold energy measurements noticeably (with uncertainties of ±0.02 eV). Accordingly, Majdi et al.[28] assigned no vibrational structure to hot bands in their photoelectron spectra of thymine recorded in similar supersonic helium beam conditions as our experiments. Therefore we propose that the thresholds shown in Fig. 4 correspond to producing electronically excited thymine and uracil with exactly the additional vibrational energy required to dissociate the respective radical cations in their I_0 states.

If the interpretation above is correct, we can deduce the adiabatic energies of the electronic excited states that absorb the ionizing photon: (5.55±0.02 - 1.88±0.06 =) 3.67±0.07 eV for thymine and (5.57±0.02 - 1.80±0.06 =) 3.77±0.07 eV for uracil. These values are in good agreement with the most recent calculations (at RI-CC2/aug-cc-pVDZ level[26]) for the S_1 states: 3.72 eV for thymine (earlier 3.73-4.03 eV at various levels of theory[30]) and 3.69 eV for uracil (earlier 3.74-4.04 eV[30]). By contrast, the present values are clearly lower than the calculated adiabatic energies for S_2 (4.49 eV for thymine[9] and 4.48 eV for uracil [31]) and higher than those for T_1 (2.84-3.16 eV for thymine and 2.87-3.31 eV for uracil[9,26,30]). Aside from S_1, the only calculated band origins that are close to the present values correspond to the T_2 states: 3.84-3.93 eV for thymine[9] and 3.84-3.91 eV for uracil[30]. He et al.'s[7] pump (267 nm) - probe measurements showed that hydration significantly suppressed access to the long-lived dark states of thymine and methyl-substituted uracil. However hydration does not have a significant suppressive effect on the MPI signals from thymine[18] and uracil[12] using the present experimental system. This suggests that ionization from triplet states did not contribute significantly to the present results.

To our knowledge, the only previous experimental adiabatic energies of uracil and thymine's low-lying electronic excited states were reported by Tsuchiya et al.[32]. They identified two bands of vibronic peaks in the fluorescence spectra of each nucleobase and attributed these to the S_1 states of different tautomers. The origins of the lower-energy vibronic band for each molecule (3.833 eV for uracil and 3.857 eV for thymine) are close to the values deduced from the present analysis (the differences are of the order of a single quantum of C=O stretching[32]) and also to the calculated S_1 adiabatic energies of the diketo tautomers. Hence the most recent calculations suggest that the lower-energy vibronic bands are traced to diketo tautomers, whereas Tsuchiya et al.[32] assigned them to unspecified keto-enol forms. It is worth noting that ionization of a given S_1 tautomer state does not imply that metastable HNCO loss took place from the equivalent tautomer of the radical cation. The high level of vibrational excitation (≥1.8 eV) of the radical cations in the present experiments means that numerous tautomeric transitions (barriers ~1 eV[33]) can be expected in the microseconds prior to

dissociation.

The count rate of the metastable channel is significantly higher in the thymine measurements than the uracil data. Indeed, the total MPI signal from thymine is approximately five times greater than from uracil in the present experiments. Uracil and thymine are similar in terms of their vapor pressures[34], their calculated photoabsorption cross-sections, and the vertical energies of their S_1 and S_2 states[30,35]. Therefore we attribute thymine's relatively high MPI efficiency to the combined effect of lower ionization thresholds (e.g. AIE = 8.82±0.03 eV compared with 9.15±0.03 eV for uracil[21]) and slower relaxation from its low-lying singlet states (average exponential decay time constant following 267 nm photo-excitation = 6.4 ps compared with 2.4 ps for uracil[36]).

4.   Conclusions

Strong signals for metastable HNCO loss from multi-photon ionized thymine and uracil molecules have been observed with clear photon energy thresholds. We do not observe threshold behavior of this kind in the signals of any fragment ion channels that can be produced efficiently by direct excitation from neutral electronic ground state, notably in electron impact ionization. Therefore the present thresholds are strongly indicative of critical processes in neutral excited states. More specifically, we propose that they can be attributed to accessing the neutral molecules' S_1 states with vibrational energy matching the threshold for HNCO loss from the radical cation. If this interpretation is correct, the present results can be combined with existing photoionization energies to deduce the excited states' adiabatic energies: 3.67±0.07 eV for thymine and 3.77±0.07 eV for uracil. These values are close to the origins of the lowest-lying vibronic bands observed in previous LIF spectra and are in good agreement with the most recent calculated adiabatic energies of the diketo S_1 states. More generally, the present results suggest that the study of metastable fragment ion production in MPI experiments has considerable potential to provide insights into the dynamics of molecules in high vibronic states.

Acknowledgements

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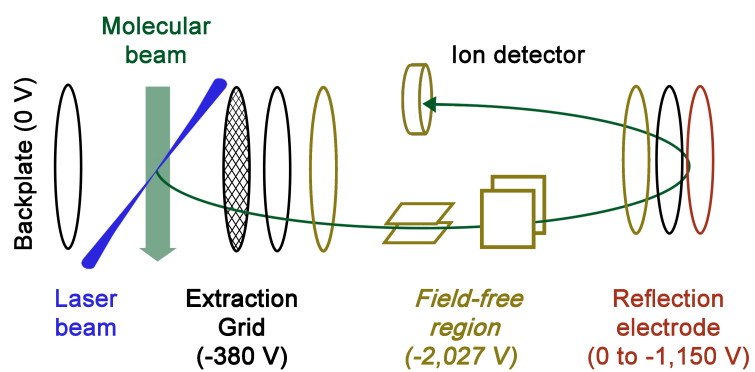**Figure 1:** Schematic diagram of the reflectron mass spectrometer.

Figure 2: MPI (220 nm, average fluence 9×10^7 Wcm^{-2}, He 0.8 bar) time-of-flight contour plots of (a) thymine and (b) uracil as a function of the reflection voltage. Selected bands are labeled in accordance with assignments by Jochims et al.[21] and Ryszka et al.[13]. The strong metastable bands in plots (a) and (b) extend to reflection voltages of -830±1 and -907±1 V, respectively.
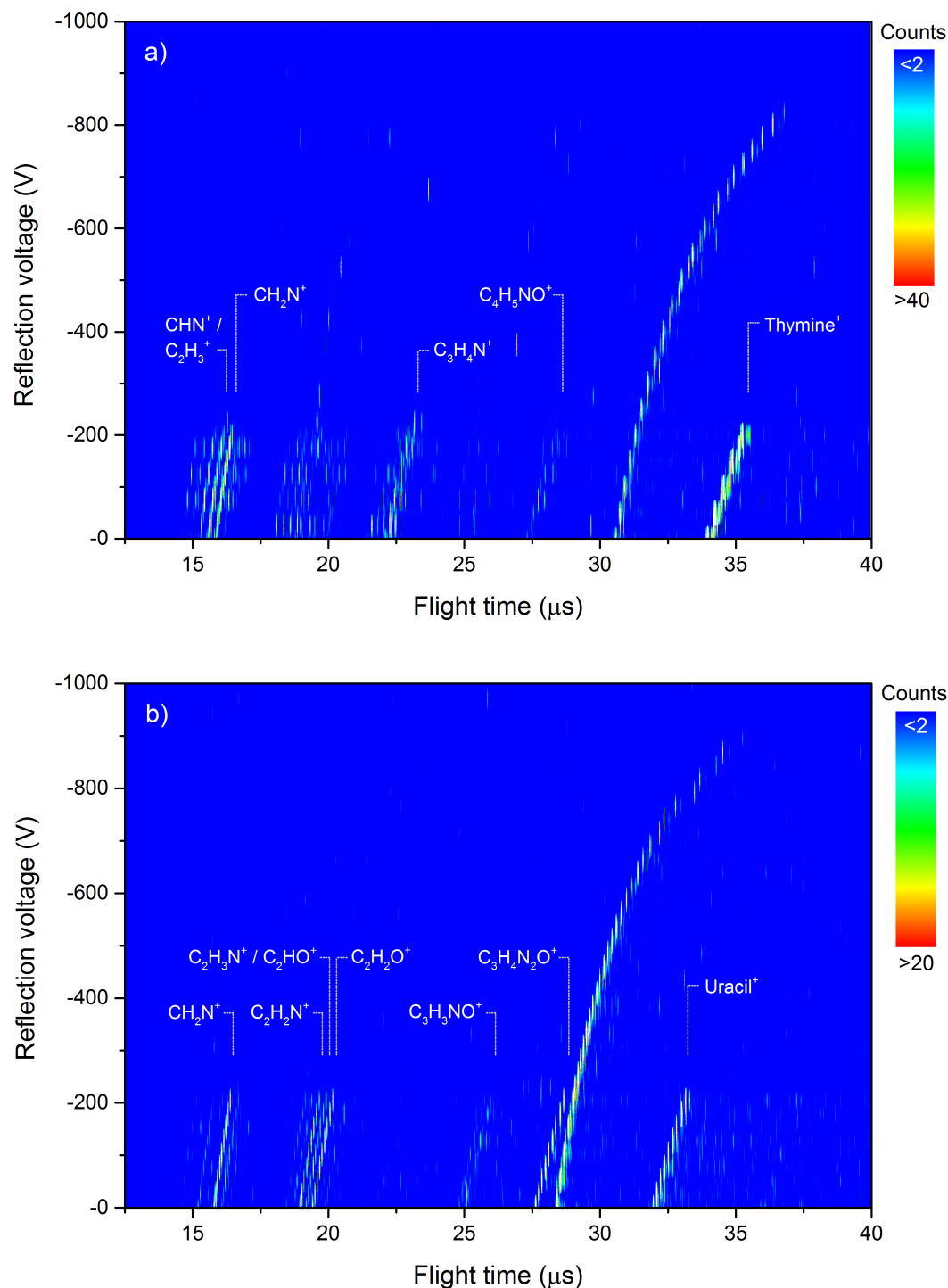
Figure 3: Power dependence (α) for metastable $C_4H_5NO^+$ production on laser pulse energy (average fluence 5-9$\times$10^7 Wcm^{-2}, 220 nm, He 0.6 bar). As α(*meta* $C_4H_5NO^+$) = 1.4$\pm$0.2 matches α(thymine$^+$) = 1.7+0.3 from the same measurements to within the uncertainties, this result supports metastable $C_4H_5NO^+$ production being a 2-photon process.
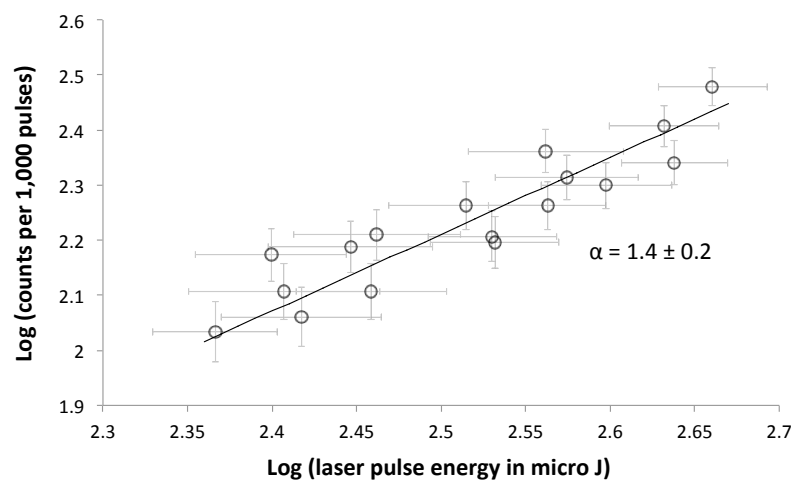
Figure 4: Photon energy dependence for metastable HNCO loss from multi-photon ionized (a) thymine and (b) uracil (220-230 nm, average fluence 9×10^7 Wcm^{-2}, He 0.8 bar). The metastable HNCO loss signals are shown as percentages of the intact parent ion signals. The presently calculated structures of the radical cations are shown schematically. Arani et al.[22] proposed that the encircled HNCO group is removed from uracil$^+$ and we have assumed here an analogous dissociation of thymine$^+$.
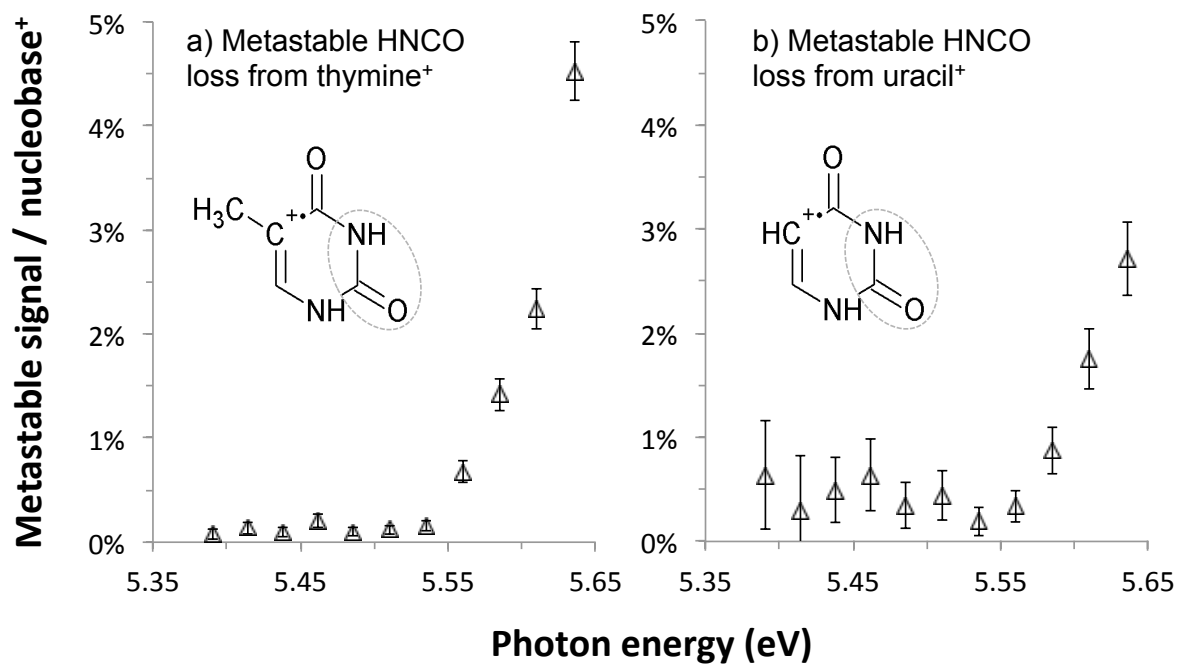
Figure 5: Comparison of MPI mass spectra of thymine at 220 and 270 nm (respective photon energies 5.64 and 4.59 eV, average fluence $9×10^7$ Wcm^{-2}, powder 250 °C, He 0.8 bar, reflection voltage -80 V). The strongest peaks observed in single photon ionization experiments are labeled with the previous assignments[21] and show no evidence for threshold behavior. Metastable HNCO loss from thymine^{+*} is also labeled in the 220 nm measurement but the mass calibration does not apply to this feature.