Photoinduced C—I bond homolysis of 5-iodouracil: A singlet predissociation pathway

Xiaojuan Dai,[1,2] Di Song,[1,a)] Kunhui Liu,[1,3] and Hongmei Su[1,2,3,a)]

[1]*Beijing National Laboratory for Molecular Sciences (BNLMS), Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China*
[2]*University of Chinese Academy of Sciences, Beijing 100049, China*
[3]*College of Chemistry, Beijing Normal University, Beijing 100875, China*

5-Iodouracil (5-IU) can be integrated into DNA and acts as a UV sensitive chromophore suitable for probing DNA structure and DNA-protein interactions based on the photochemical reactions of 5-IU. Here, we perform joint studies of time-resolved Fourier transform infrared (TR-FTIR) spectroscopy and *ab initio* calculations to examine the state-specific photochemical reaction mechanisms of the 5-IU. The fact that uracil (U) is observed in TR-FTIR spectra after 266 nm irradiation of 5-IU in acetonitrile and ascribed to the product of hydrogen abstraction by the uracil-5-yl radical (U·) provides experimental evidence for the C—I bond homolysis of 5-IU. The excited state potential energy curves are calculated with the complete active space second-order perturbation//complete active space self-consistent field method, from which a singlet predissociation mechanism is elucidated. It is shown that the initially populated $^1(\pi\pi^*)$ state crosses with the repulsive $^1(\pi\sigma^*)$ or $^1(n_I\sigma^*)$ state, through which 5-IU undergoes dissociation to the fragments of (U·) radical and iodine atom. In addition, the possibility of intersystem crossing (ISC) is evaluated based on the calculated vertical excitation energies. Although a probable ISC from $^1(\pi\pi^*)$ state to $^3(n_O\pi^*)$ and then to the lowest triplet $^3(\pi\pi^*)$ could occur in principal, there is little possibility for the excited state populations bifurcating to triplet manifold, given that the singlet state predissociation follows repulsive potential and should occur within dozens to hundreds of femtoseconds. Such low population of triplet states means that the contribution of triplet state to photoreactions of 5-IU should be quite minor. These results demonstrate clearly a physical picture of C—I bond homolysis of 5-IU and provide mechanistic illuminations to the interesting applications of 5-IU as photoprobes and in radiotherapy of cancer. *Published by AIP Publishing.* [http://dx.doi.org/10.1063/1.4973650]

I. INTRODUCTION

5-Halouracils (5-iodouracil or 5-bromouracil) are analogues of thymine where the methyl group of thymine is substituted by an iodine or bromine atom. Because of the similarity in molecular structure and size, 5-halouracils can replace the thymine base of DNA. Interestingly, such replacement does not impair the functionality of DNA *in vivo* but enhances the UV sensitivity of the cell, accelerating DNA damage and cell death upon ionizing radiation or UV photolysis.[1–5] Hence, 5-halouracils can serve as potential sensitizers for radiotherapy of cancer. On the other hand, UV sensitization of 5-halouracils can induce DNA strand breaks and DNA-protein cross-linking, acting as an integrated chromophore excellent for probing DNA structure and investigating specific DNA-protein interactions.[6–10] Due to the biological and medicinal importance,[6–13] 5-halouracils have attracted continuous research interests.

Investigations have shown that these sophisticated applications generally start with the photochemical reactions of the chromophore (5-halouracils, Scheme 1).[3,9,14–16] Understanding the photochemical reaction mechanisms of the chromophore is, therefore, essential for elucidation of the mechanisms of DNA strand breaks and DNA-protein

cross-linking. For 5-bromouracil (5-BrU), the photoactive properties have been substantially explored,[17–20] which provides fundamental information for reasonably interpreting strand breaks and photo-cross-linking induced by 5-BrU-containing DNA. Earlier studies indicated that C—Br bond homolysis takes place in the higher energy $\pi\pi^*$ singlet state; little or no bond homolysis occurs in the lower energy $n\pi^*$ singlet state. After intersystem crossing (ISC), the triplet state is populated.[17,19] In this case, a photoinduced electron transfer mechanism was raised to rationalize the 5-BrU induced DNA strand breaks and DNA-protein cross-linking, where the sequential reactions start with photoinduced electron transfer from an adjacent purine base or an aromatic amino-acid residue to the triplet 5-BrU unit, followed by loss of a bromine anion and generation of the uracil-5-yl (U·) radical that cause cross-linking with DNA or protein.[3,21,22] As the excitation energy further increases, the mechanism of C—Br bond homolysis to (U·) radical may be also involved in the DNA strand breaks and DNA-protein cross-linking reactions.[9,20]

As for 5-iodouracil (5-IU), due to the weaker C—I bond, its primary photochemical process is believed to be carbon-iodine (C—I) bond homolysis[23,24] similar to other organic iodides,[25–32] including vinyl iodides.[33] As a result, it is expected that 5-IU-containing DNA strand breaks and DNA-protein cross-linking reactions could be trigged by C—I

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a)Authors to whom correspondence should be addressed. Electronic addresses: hongmei@iccas.ac.cn and songdi@iccas.ac.cn
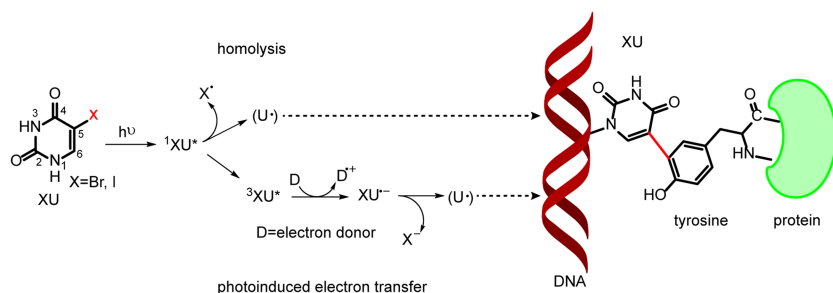
SCHEME 1. Proposed photochemical mechanisms for the 5-halouracils causing DNA–protein cross-linking (with the cross-linking between 5-halouracil and tyrosine as an example).

bond homolysis.[14,16] However, for some DNA strand breaks reactions such as the duplex DNA containing 5′-G(A)$_n$IUT-3′ sequence, photoinduced electron transfer from an adjacent purine base to the excited 5-IU unit was assumed to coexist with the C—I bond homolysis,[15] and for some macromolecular DNA-protein cross-linking reactions, photoinduced electron transfer seemed to be also necessary to account for high cross-linking yields.[10,16] It follows that the molecular mechanisms underlying the interesting photochemical applications of 5-IU remain obscure.

To clarify these issues, it is essential to understand the photochemical reaction mechanisms of 5-IU. However, only a few photochemical studies were performed with UV lamp dating back to 1965[23,24] and with high performance liquid chromatography (HPLC), NMR, and photoacoustic calorimetry in 1996.[16] In regard to the limited knowledge for 5-IU, we perform time-resolved IR spectroscopy experiments in conjunction with *ab initio* quantum-chemical calculations to examine further the state-specific mechanisms of the 5-IU photochemistry. Upon 266 nm laser photolysis of 5-IU in acetonitrile, U was observed in the time-resolved IR spectra and was ascribed to the hydrogen abstraction product of the (U·) radical following the primary C—I bond homolysis. In combination with the complete active space second-order perturbation (CASPT2)// the complete active space self-consistent field (CASSCF) calculations of excited state dissociation potential energy curves (PECs), a singlet state predissociation mechanism is elucidated. Two dissociative states, $^1(\pi\sigma^*)$ and $^1(n_I\sigma^*)$, which can couple with the initially populated $^1(\pi\pi^*)$ state in the Franck-Condon (FC) region, are found to be the protagonists of C—I bond homolysis in the singlet state of 5-IU generating (U·) radical. Given that the singlet photodissociation pathway follows repulsive potential and should occur rapidly within dozens to hundreds of femtoseconds, there is little possibility for the excited state populations bifurcating to triplet manifold, thus indicating a predominant singlet C—I bond homolysis mechanism and quite minor contribution of triplet state to photochemical reactions of 5-IU. These results demonstrate clearly a physical picture of the photoinduced C—I bond homolysis of 5-IU and provide mechanistic illuminations to the interesting applications of 5-IU as photoprobes and in radiotherapy of cancer.

II. EXPERIMENTAL AND COMPUTATIONAL METHODS

A. Materials

5-Iodouracil (J&K chemicals, 99%) and uracil (Acros Organics, 99%) were used as received. HPLC grade acetonitrile (CH$_3$CN) was used as solvent.

B. Time-resolved Fourier transform IR (TR-FTIR) experiment

Nanosecond time-resolved infrared absorption spectra were measured using step-scan, time-resolved Fourier transform infrared (TR-FTIR) experimental setup, combined with an Nd:YAG laser (Surelite II, Continuum). Step-scan FTIR spectrometers are commercially available but require significant modification for the applications in a flash photolysis time-resolved infrared study. Detailed experimental procedures for TR-FTIR absorption spectroscopy[34,35] have been described in our previous publications.[36] Briefly, the TR-FTIR instrument is comprised by a Nicolet Nexus 870 step-scan FTIR spectrometer, a Continuum Surelite II Nd YAG laser, and a pulse generator (Stanford Research DG535) to initiate the laser pulse and achieve synchronization of the laser with data collection, two digitizers (internal 100 KHz 16-bit digitizer and external 100 MHz 14-bit GAGE 14100 digitizer) which offer fast time resolution and a wide dynamic range as needed, and a personal computer to control the whole experiment. The detector used in this work is the photovoltaic MCT (0.5 mm) equipped with a fast internal preamplifier (50 MHz).

The fourth harmonic of Nd: YAG laser (266 nm) operating at 10 Hz repetition rate was used in the experiments. The laser excitation beam was directed through an iris aperture (3 mm in diameter) and then overlapped with the infrared beam in the sample cell within the sample compartment of the FTIR spectrometer. The laser beam energy after the aperture was 2 mJ per pulse. The IR spectra were collected with a spectral resolution of 8 cm^{-1}. A Harrick flowing solution cell with 2 mm thick CaF$_2$ windows (path-length, 500 μm) was used for the measurements. The closed flowing system is driven by a peristaltic pump (ColeParmer Masterflex) to refresh the sample before every laser pulse.

C. Theoretical calculations

In aid of assignments of IR spectra, ground state geometries and IR vibrational frequencies for the reactant 5-IU and possible product species were calculated using the density functional theory (DFT) B3LYP method with the 6-311+G (d, p) basis sets for C, N, O, and H atoms, and lanl2dz basis set for I atom,[37,38] which has proved to be a sufficient and affordable computational method for the current system.[39,40] To simulate the effect of the solvent (CH$_3$CN) on 5-IU, the polarized continuum model (PCM) was used in the calculations.[41,42]

To describe the photodissociation process of 5-IU, excited state *ab initio* calculations were performed using the CASPT2//CASSCF approach. The ground state geometry of

5-IU was optimized by the CASSCF (complete active space self-consistent field) method, and energies were refined at the CASPT2 (complete active space second-order perturbation) level to account for dynamic correlation not included in the CASSCF calculation. The basis set of 6-31+G* was employed on the C, N, O, and H atoms, in association with the lanl2dz basis set for I atom. Other basis sets of 6-31G and aug-cc-pVDZ for C, N, O, and H atoms were also tested. By comparing calculated vertical energies with experimental values, it is found that the 6-31+G* results are the best in agreement with the experimental UV-Vis spectra of 5-IU. We then adopt the 6-31+G* results in the following discussions.

The active space, shown in Fig. S1 of the supplementary material, involves 16 electrons distributed in 12 orbitals, referred to as CASPT2//CASSCF (16, 12). Specifically, the 12 orbitals comprise three aromatic ring π orbitals, two π orbitals of oxygen atom, a π orbital and a non-bonding (n) orbital of iodine atom, a C—I σ orbital, three π^* antibonding orbitals, and a C—I σ^* antibonding orbital. To compute the $^1(n_O\pi^*)$ and $^3(n_O\pi^*)$ transition energies (excitation from the oxygen lone pairs), the CASPT2 calculations were repeated with the (16, 12) active space where the n orbital of iodine atom is replaced with that of O atom nearest to I atom. For each active space, five singlet roots were calculated with a state-averaged CASSCF procedure. To minimize the presence of intruder states, an imaginary level-shift correction of 0.3 a.u. was applied in the CASPT2 calculations.[43]

The DFT calculations were performed with the Gaussian 09 program package,[44] while the CASPT2//CASSCF calculations were implemented with MOLPRO 2008.1 package.[45]

III. RESULTS AND DISCUSSION

A. TR-FTIR spectroscopy measurement of the photoproducts

Due to the specific vibrational marker bands and the IR discerning capability, time-resolved infrared spectroscopy is a powerful technique to directly probe the photochemical reactions.[39,46–48] By means of time-resolved Fourier transform infrared spectroscopy (TR-FTIR), we measured 266 nm photolysis of 5-IU in CH$_3$CN solution under the N$_2$-saturated conditions to prevent the complexity brought by oxidation when O$_2$ is present (Fig. S2 and Scheme S1 in the supplementary material). The excitation wavelength of 266 nm is used because 5-IU absorbs strongly at 266 nm in the UV-Vis spectrum (Fig. S3 in the supplementary material).

As shown in the TR-FTIR spectra (Fig. 1(b)), immediately after UV excitation, three intense bleaching bands at 1685, 1724, and 1758 cm^{-1} and a noise level bleaching band at 1624 cm^{-1} were observed, which should be ascribed to the depletion of ground state 5-IU molecule. The four identical bands also appeared in the steady-state IR absorption spectrum at 1622, 1689, 1723, and 1754 cm^{-1} for 5-IU (Fig. 1(a)), confirming the assignment. To further attribute these absorption bands of 5-IU, IR frequencies and intensities of 5-IU were calculated with the B3LYP method (Table I). The calculated IR frequencies of 1644 cm^{-1} (C=C stretching vibration), 1699 cm^{-1} (C4=O stretching vibration) for 5-IU
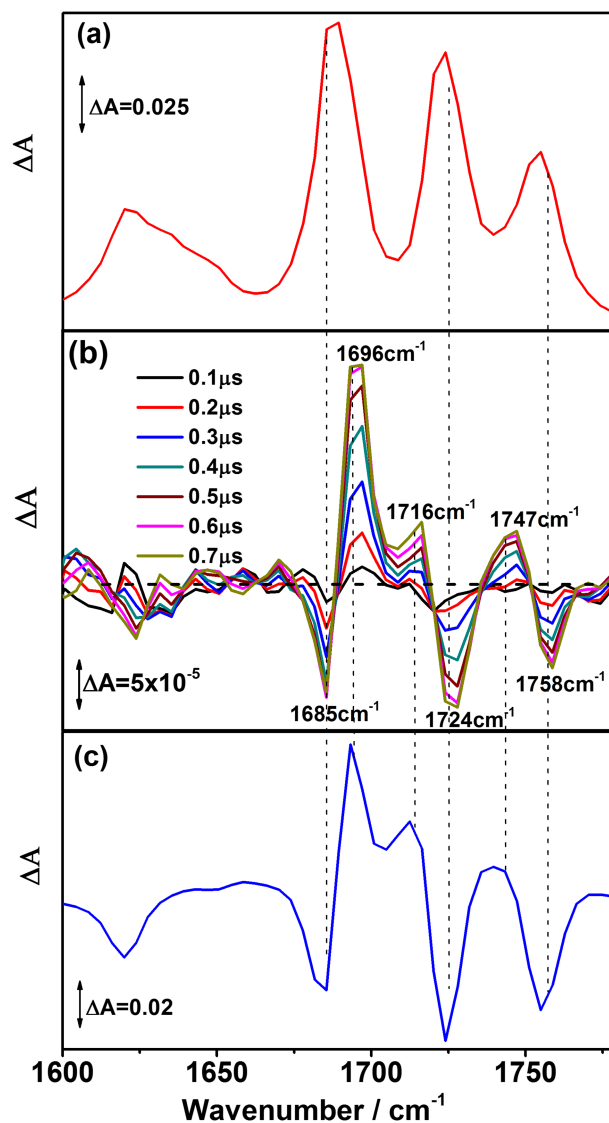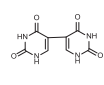

FIG. 1. (a) Steady-state IR spectrum of 2.7 mM 5-IU in CH$_3$CN; (b) infrared transient absorption spectra of 2.7 mM 5-IU in CH$_3$CN solution at selected time delays following 266 nm laser irradiation under N$_2$-saturated condition; (c) steady-state IR difference spectrum of 2.7 mM 5-IU CH$_3$CN solution after 1 min of 266 nm laser irradiation.

in CH$_3$CN agree well with the observed peaks at 1622 and 1689 cm^{-1} in its steady-state IR spectrum (Fig. 1(a)); thus, the two absorption peaks at 1622 and 1689 cm^{-1} correspond to the C=C and C4=O stretching vibration of 5-IU, respectively. The two bands measured at 1723 and 1754 cm^{-1} should be resulted from the splitting of C2=O mode (calculated to be 1747 cm^{-1}) due to Fermi-resonance of fundamentals with combination bands.[49,50]

The ground state bleaching bands are accompanied by the formation of three positive bands at 1696, 1716, and 1747 cm^{-1} in the TR-FTIR spectra (Fig. 1(b)). These positive bands should arise from the transient intermediates or stable products of the photochemical reactions of 5-IU. These positive bands build up gradually from 100 ns to 700 ns, indicating that they are most likely ascribed to photochemical reaction products instead of the triplet 5-IU, because the triplet lifetime is as short as <100 ns[51] and should not result in transient signals at times longer than 100 ns. According to our experimental

TABLE I. B3LYP/6-311+G(d,p) (lanl2dz basis set for I atom) calculated IR frequencies (cm^{-1}) and IR Intensities for the possible relevant species in the photochemical reaction of 5-IU in CH$_3$CN with the solvation effect simulated by the PCM model.[a]

		Mode	Frequency (cm^{-1})	Intensity (km mol^{-1})
5-IU		C5=C6	1644	435
		C4=O	1699	1245
		C2=O	1747	1302
(U·)		C5=C6	1654	287
		C4=O	1683	1288
		C2=O	1740	924
U		C5=C6	1660	232
		C4=O	1692	1808
		C2=O	1744	935
U-U		C5=C6	1643	760
		C4=O	1682	2115
		C2=O	1736	3184

[a]Only the vibrational modes with considerable IR intensities are listed here.

conditions, possible photochemical reactions of 5-IU are proposed in Scheme 2. It is expected that 5-IU primarily undergoes C—I bond homolysis after the nanosecond 266 nm laser irradiation, leading to the formation of (U·) radical and iodine atom (Eq. (1), Scheme 2). (U·) radical is a significant transient species, which is the key to invoke further DNA-protein cross-linking and DNA strand breaks when 5-IU used as photoprobes or in radiotherapy of cancer. Under N$_2$ saturation condition without oxidation involved, transient (U·) radical further converts into stable product U by hydrogen abstraction from the solvent CH$_3$CN used in our TR-FTIR experiment (Eq. (2), Scheme 2), since CH$_3$CN is a pure hydrogen atom donor with no expected influences on triplet state or electron transfer chemistry.[16] Another possible decay pathway is the radical-radical recombination, leading to stable product 5,5′-diuracil (U-U) (Eq. (3), Scheme 2). Because (U·) radical is surrounded by a large amount of CH$_3$CN, the hydrogen abstraction to produce U is predominant over the radical-radical recombination forming U-U dimer.

For relevant transients and stable products involved in Scheme 2, we calculated their IR frequencies and intensities. As shown in Table I, the predicted vibrational frequencies of C4=O and C2=O stretching modes at 1683 cm^{-1} and 1740 cm^{-1} for (U·) radical, 1692 cm^{-1} and 1744 cm^{-1} for U, and 1682 cm^{-1} and 1736 cm^{-1} for U-U are all close to the observed peak positions of 1696 and 1747 cm^{-1}; therefore, it appears at first glance that they are all likely to be responsible for the 1696 and 1747 cm^{-1} peaks in the TR-FTIR spectra.

Further, we analyzed decay kinetics of the three positive peaks (Fig. 2), for the purpose of distinguishing the transients and stable products. After fast formation within 0.7 μs, these transient positive bands sustain their intensities and decay slowly at several hundreds of μs due to the diffusion out of the observation zone with lifetime of 285 ± 39 μs, 285 ± 57 μs, and 259 ± 60 μs, respectively (Fig. 2). This means that these newly formed positive bands should mostly arise from stable products rather than the short-lived transient (U·) radical. From this, it can be inferred that the process of (U·) radical converting to the stable products is very rapid such that no transient radical is detected. Moreover, the steady-state IR difference spectrum after UV-irradiation (Fig. 1(c)) also reveals identical
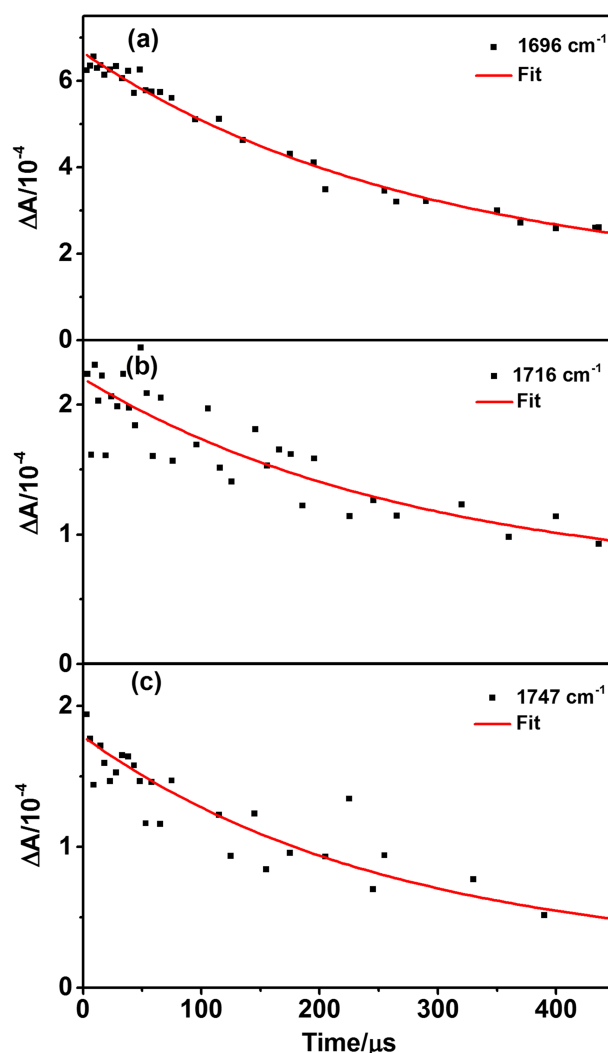
SCHEME 2. Possible photochemical reactions following the photolysis of 5-IU in CH$_3$CN solution under N$_2$-saturated condition.

FIG. 2. Kinetic traces for the positive photoproduct bands fitted with single-exponential decay functions: (a) at 1696 cm^{-1}, (b) at 1716 cm^{-1}, (c) at 1747 cm^{-1}.

bands at these positions, confirming further the three positive bands should be assigned to the stable products, in this case, the secondary photoproducts U or U-U.

To clarify the assignment of the stable products, we compared the calculated IR intensities of U and U-U with the experimental intensities of the positive peaks respectively. For U-U, C4=O stretching vibration (1682 cm^{-1}) is obviously weaker than the calculated C2=O stretching vibration (1736 cm^{-1}), on the basis of calculated IR intensities listed in Table I. Such a characteristic IR intensity feature for U-U is different from what is observed in the TR-FTIR spectra (Fig. 1(b)). In the TR-FTIR spectra, the absorption intensity of low wavenumber band at 1696 cm^{-1} is much stronger than that of high wavenumber bands at 1747 cm^{-1}. This indicates that the contribution of U-U to the positive bands in the TR-FTIR spectra should be minor or negligible. On the contrary, for U, the predicted intensity ratio of C4=O mode (1692 cm^{-1}) to the C2=O mode (1744 cm^{-1}) is similar to the signal magnitude for the two corresponding positive peaks observed in the TR-FTIR spectra. Therefore, we can deduce that U should be mainly responsible for the two observed positive bands at 1696 cm^{-1} and 1747 cm^{-1}.

To substantiate further the assignment, the steady-state IR spectrum of the authentic sample of U in CH$_3$CN was recorded. As shown in Fig. 3, the IR spectrum of U shows strong C4=O absorption band at 1693 cm^{-1} and weak broad absorption bands at 1700-1800 cm^{-1} with two peaks at 1720 and 1744 cm^{-1} caused by Fermi resonances of C2=O fundamental vibration with some combination or overtone vibrations.[40,49] As expected, the spectral positions of U at 1693 and 1744 cm^{-1} match those observed in the TR-FTIR spectrum (1696 cm^{-1} and 1747 cm^{-1}). In addition, the absorption at 1720 cm^{-1} of U also matches well with the positive band at 1716 cm^{-1} in the TR-FTIR spectrum, corresponding to one of the Fermi Resonance peaks of C2=O vibrations for U. The intensity ratio of the three positive peaks in the TR-FTIR spectra consists with that in the IR spectrum for the authentic sample U (Fig. 3).Thus the three positive bands in the TR-FTIR should be all ascribed to the photoproduct U. The small peak shift of 3 or 4 cm^{-1} in the TR-FTIR spectrum relative to the authentic sample is resulted from the partial overlapping of the positive band of U with the negative band of 5-IU. Indeed,
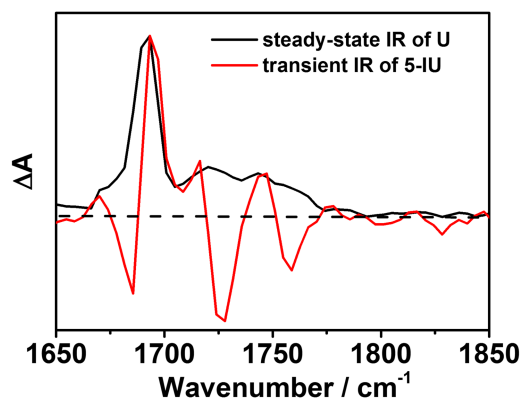
taking into account the fact that (U·) radical is surrounded by hydrogen donor solvent CH$_3$CN and the chance of hydrogen abstraction is enhanced while the recombination with another (U·) radical is hindered, it is reasonable to assign the observed stable product to U.

In brief, we observed the formation of U as the stable photoproduct for the photolysis 5-IU in CH$_3$CN solution under N$_2$ saturation in the TR-FTIR spectra. Since the solvent CH$_3$CN is a pure hydrogen atom donor, U should be formed by the rapid hydrogen abstraction of (U·) radical, which is produced from C—I bond homolysis of 5-IU after UV photolysis. Therefore, C—I bond homolysis of 5-IU is proved experimentally.

B. CASPT2//CASSCF calculations characterizing state-specific photochemical mechanisms

To interpret the experimental observations and provide mechanistic insights, we performed excited state *ab initio* calculations using the CASPT2//CASSCF approach with the basis set of 6-31+G*-lanl2dz. The geometry of the ground state (S$_0$) of 5-IU was optimized using the CASSCF method. As shown in Fig. S4 of the supplementary material, the geometry within C1 symmetry is in accordance with early calculation results[50] and our DFT results. For the important structural parameter, the C—I bond length, the CASSCF result gives a value of 2.15 Å, which is slightly longer than the X-ray experiment value by 0.04 Å.[52] Given that the calculation was performed in gas phase for the isolated 5-IU molecule and the X-ray measurement was for the crystal sample where hydrogen bond exists between 5-IU molecules, this bond length difference between calculation and experiment is reasonable.

On the basis of the optimized ground state geometry, the vertical excitation energies of excited states of 5-IU were calculated and the data are collected in Table II. The calculations reveal clearly the transition properties of five excited singlet states to be πσ*, ππ*, n$_I$σ*, n$_O$π*, and π$_I$σ* (see Table II). As shown in Table II, the lowest excited singlet state locates vertically above S$_0$ at 4.44 eV, which corresponds to πσ* transition from the occupied π orbital of the C=C bond to the antibonding σ* orbital of the C—I bond. The second excited singlet state at 4.69 eV is the ππ* transition within the C=C bond. The next higher state, ^{1}(n$_O$π*), locates vertically at 4.85 eV, with the electron promoted from the nonbonding n orbital of the O atom of the C4=O double bond to the antibonding π*orbital of the C=C bond. In addition, there are two even higher lying states, ^{1}(n$_I$σ*) state at 5.03 eV and ^{1}(π$_I$σ*) state at 6.14 eV

FIG. 3. The steady-state IR spectrum of U in CH$_3$CN (black line) and one time slice of the TR-FTIR spectra for 5-IU following 266 nm laser irradiation (red line). The intensity of the two spectra is normalized for comparison.

TABLE II. Vertical excitation energies and oscillator strengths (f) for 5-IU calculated at CASPT2//CASSCF (16, 12)/6-31+G* (lanl2dz for I atom) level of theory.

Singlet states			Triplet states	
State	E$_{exc}$ (eV)	f	State	E$_{exc}$ (eV)
^{1}(πσ*)	4.44	0.0050	^{3}(ππ*)	3.56
^{1}(ππ*)	4.69	0.3169	^{3}(π$_I$π*)	3.87
^{1}(n$_O$π*)	4.85	0.0005	^{3}(n$_I$σ*)	4.27
^{1}(n$_I$σ*)	5.03	0.0008	^{3}(n$_O$π*)	4.67
^{1}(π$_I$σ*)	6.14	0.0005	^{3}(π$_O$π*)	5.38

which correspond to excitation of the n orbital and π orbital to the σ^* orbital of C—I bond, respectively.

Further, we analyzed the oscillator strength (f) for transitions to the five excited singlet states. The $^1(\pi\sigma^*)$, $^1(n_O\pi^*)$, $^1(n_I\sigma^*)$, and $^1(\pi_I\sigma^*)$ states all have approximately zero oscillator strength, whereas only the $^1(\pi\pi^*)$ state has a considerable f value of 0.3169. Obviously, only $^1(\pi\pi^*)$ state is spectroscopically bright and can be reached by laser excitation. Indeed, 5-IU exhibits an absorption band with maximum at 275 nm (4.52 eV) in the UV-Vis absorption spectrum (Fig. S3 in the supplementary material), which coincides with the calculated $^1(\pi\pi^*)$ vertical energy of 4.69 eV. The good agreement between the experimental result and the calculated energy demonstrates the sufficient accuracy of the current level of calculation.

As discussed above, the bright $^1(\pi\pi^*)$ is the initially populated state upon 266 nm excitation, the laser wavelength used in our experiment. To reveal the C—I bond homolysis mechanism, we calculated the potential energy curves (PECs) of several singlet excited states in the vicinity of the $^1(\pi\pi^*)$ state. According to previous ultrafast time-resolved studies, the dissociations of many iodine-containing species such as methyl iodide, iodobenzene, and 4-iodobiphenyl were observed to occur on a time scale of dozens to hundreds of femtoseconds,[53,54] showing that the dissociation time of the C—I bond should be shorter than the rotational period of parent molecules and most geometry parameters remain unchanged during dissociation.[55] For this reason, the geometry of the skeleton, the N-containing aromatic ring of 5-IU, was fixed and only the dissociation reaction coordinate C—I bond length was varied in the calculations of PECs.

Fig. 4 shows the PECs along the C—I bond distance (R_{C-I}) for the ground state and the four singlet excited states, $^1(\pi\sigma^*)$, $^1(\pi\pi^*)$, $^1(n_I\sigma^*)$, and $^1(\pi_I\sigma^*)$. Starting from the bright state $^1(\pi\pi^*)$, three photodissociation pathways leading to the (U·) radical and I atom can be elucidated. In the first pathway, a facile internal conversion (IC) from the $^1(\pi\pi^*)$ state to the lowest excited state of $^1(\pi\sigma^*)$ is predicted because of small energy gap of the two states in the FC region. The $^1(\pi\sigma^*)$ potential is repulsive, corresponding to a barrierless dissociation of the C—I bond. As shown in Fig. 4, the $^1(\pi\sigma^*)$ state and the ground state both converge to the same dissociate limit, meaning that
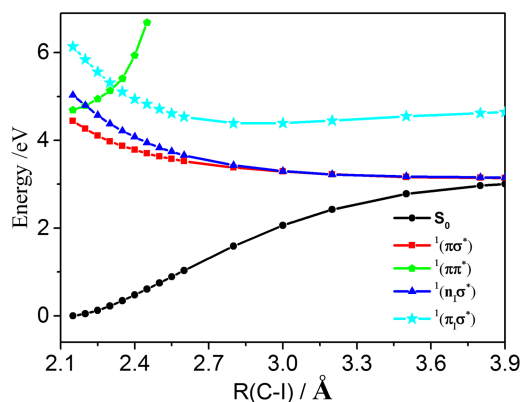

FIG. 4. The potential energy curves for five singlet states of 5-IU calculated at the CASPT2//CASSCF (16, 12)/6-31+G* (lanl2dz basis set for I atom) level of theory.

they have the same products, the (U·) radical and I atom. In the second pathway, the $^1(\pi\pi^*)$ state crosses to another repulsive state $^1(n_I\sigma^*)$, which can also barrierlessly dissociate into the (U·) radical and I atom. The crossing point occurs at R_{C-I} of 2.20 Å, which is 4.79 eV above the S_0 minimum. At a longer C—I bond distance of 2.30 Å, the $^1(\pi\pi^*)$ state crosses with another higher repulsive state $^1(\pi_I\sigma^*)$ at a vertical excitation energy of 5.25 eV, leading to the excited (U·) radical and I atom. The predissociation via the higher $^1(\pi_I\sigma^*)$ state is thus the third pathway.

According to the calculations, the first and second pathways only require excitation energies of 4.69-4.79 eV as their crossing points are low in energy, whereas the third pathway requires higher excitation energy (5.25 eV) to overcome an energy barrier for reaching the crossing points. For our experiment, the laser wavelength used is 266 nm, which can offer an excitation energy of about 4.66 eV. This energy is sufficient to allow the transition from $^1(\pi\pi^*)$ to $^1(\pi\sigma^*)$ or $^1(n_I\sigma^*)$, but cannot reach the crossing point of $^1(\pi\pi^*)/^1(\pi_I\sigma^*)$. Consequently, the dissociation can proceed along the first and second pathways, whereas the third pathway is energetically inaccessible. Thus, a predissociation mechanism of $^1(\pi\pi^*)$ via $^1(\pi\sigma^*)$ or $^1(n_I\sigma^*)$ can be established for the photoinduced C—I bond homolysis. Similar to other iodine-containing species, electron promotion to σ^* orbitals results in dissociative excited states and plays important roles in the photochemistry.[56,57] Such a state-specific mechanism can rationalize the experimental observations. Specifically, upon excitation at 266 nm, the $^1(\pi\pi^*)$ of 5-IU couples to the repulsive $^1(\pi\sigma^*)$ or $^1(n_I\sigma^*)$ state, dissociating into (U·) radical and I atom, and then (U·) radical abstracts the hydrogen atom of CH_3CN to form the stable product U.

In addition, the vertical energies of several low lying triplet states and the spin-orbit coupling (SOC) constant were also calculated to evaluate the possibility of ISC. As shown in Table II, the energy of $^1(\pi\pi^*)$ is almost identical to that of $^3(n_O\pi^*)$ (4.67 eV above S_0), and the calculated SOC value is 26.2 cm^{-1}, which can enable ISC process from $^1(\pi\pi^*)$ to $^3(n_O\pi^*)$. Subsequently, $^3(n_O\pi^*)$ can relax to the lowest triplet state through IC. The lowest triplet state is of the $\pi\pi^*$ character, in good agreement with the experimental postulations.[58] Meanwhile, because ISC from singlet (nπ^*) state to triplet manifold had been reported in some cases, we also pay attention to $^1(n_O\pi^*)$. But ISC from $^1(n_O\pi^*)$ is difficult to occur because of the large energy gaps between $^1(n_O\pi^*)$ and the nearby triplet states of $^3(\pi\pi^*)$, $^3(\pi_I\pi^*)$, and $^3(\pi_O\pi^*)$.

Although the ISC process from $^1(\pi\pi^*)$ to $^3(n_O\pi^*)$ and then to the lowest $^3(\pi\pi^*)$ could occur in principal, it is noted that very low population of triplet 5-IU should be yielded. According to earlier reports, the initially reached excited state $^1(\pi\pi^*)$ undergoes mainly the deactivation by IC to the ground state, as is the case for virtually all other uracil derivatives.[59,60] Meanwhile, our calculations reveal that the $^1(\pi\pi^*)$ state crosses with the repulsive $^1(\pi\sigma^*)$ or $^1(n_I\sigma^*)$ state in FC region and undergoes instantaneous C—I bond homolysis within the rotational period. This photodissociation process thus inevitably consumes the population of $^1(\pi\pi^*)$ and further decreases the possibility of ISC. This prediction is in accordance with the estimation based on the phosphorescence measurement of

5-IU in glassy media, which reported a phosphorescence yield as low as 0.03.[18] Such low population of triplet states means that the contribution of triplet state to photoreactions of 5-IU should be quite minor.

When 5-IU was incorporated into DNA as a UV sensitive chromophore to induce DNA strand breaks or DNA-protein cross-linking, as indicated from the previous studies, the photoinduced electron transfer mechanism from an adjacent purine base or amino acid residue to the excited 5-IU unit was assumed to coexist with the C—I bond homolysis.[10,15,16] In principal, photoinduced electron transfer can occur with the triplet or singlet excited 5-IU. In light of our calculations, a minimal contribution of electron transfer via triplet state is expected due to the negligible population of triplet state. If the photoinduced electron transfer mechanism is involved, it should rather proceed through the excited singlet state of 5-IU, but the occurrence of this reaction requires a strong electron donor (e.g., purine base) with appropriate conformation in close proximity to 5-IU chromophore such that no diffusion is needed and the electron transfer could complete in the time scale of dozens to hundreds of femtoseconds. Only in these circumstances, photoinduced electron transfer could compete with the singlet state barrierless C—I bond homolysis and play roles in the subsequent DNA strand breaks or DNA-protein cross-linking applications.[15,16] On the other hand, in most model systems when diffusion is required to bring 5-IU and another nucleobase or amino acid together, there is very little possibility for the photoinduced electron transfer to take place.[16]

Overall, the photochemistry of 5-IU should be dominated by the singlet state C—I bond homolysis mechanism, where the initially populated $^1(\pi\pi^*)$ state couples to the repulsive state $^1(\pi\sigma^*)$ and/or $^1(n_I\sigma^*)$ and invokes the C—I bond cleavage into I atom and (U·) radical. Only if 5-IU is exposed to an appropriate electron donor, the photoinduced electron transfer mechanism via the singlet state could play certain roles in the photochemistry of 5-IU. For example, in the bulk solution,[16,51] C—I bond homolysis dominates photochemistry of 5-IU, while in the double strand DNA 5′-G(A)$_n$IU-3′ sequence containing a strong electron donor of G bases and π-stacking arrangements,[15] both the electron transfer mechanism and the C—I homolysis mechanism were proposed to take effect. Unlike the (U·) radical which is still bound to DNA, the dissociated I atom is free and can diffuse away quickly, undergoing recombination with another I atom instead of reacting with DNA or protein.

IV. CONCLUSIONS

In this work, we used time-resolved FTIR spectroscopy and *ab initio* calculations to investigate the photochemical reaction mechanisms of 5-IU. The photochemistry of 5-IU is found to be dominated by the C—I bond homolysis upon 266 nm laser irradiation, as evidenced by the observation of the photoproduct uracil from the hydrogen abstraction of the uracil-5-yl radical following the primary C—I bond homolysis in the TR-FTIR spectra. Theoretically, CASPT2//CASSCF calculations of the PECs of five singlet states reveal a predissociative mechanism for the C—I bond homolysis. It

is shown that the initially populated $^1(\pi\pi^*)$ state by 266 nm excitation is a bound state and does not dissociate, but can rapidly couple to the repulsive $^1(\pi\sigma^*)$ state or $^1(n_I\sigma^*)$ state in the FC region, due to the small energy gap or surface crossing. Along the two repulsive PECs, 5-IU molecules undergo barrierless C—I bond cleavage to the fragments of uracil-5yl radical and iodine atom and this dissociation process should occur within dozens to hundreds of femtoseconds. Electron promotion to σ* orbitals results in dissociative excited states and plays important roles in the C—I bond homolysis. In addition, our calculations also illustrate the probable depopulation pathway of ISC to triplet state, but with low possibility, indicating that the contribution of triplet state to photoreactions of 5-IU should be quite minor. The state-specific mechanisms revealed here enable a deeper understanding of the 5-IU photochemistry that is of fundamental importance to the interesting applications of 5-IU as photoprobes and in radiotherapy of cancer.

SUPPLEMENTARY MATERIAL

See supplementary material for the TR-FTIR spectra of 5-IU photolysis under aerobic conditions, the active space used for the excited states calculations, UV−vis absorption spectrum of 5-IU in acetonitrile, and optimized geometry of ground state 5-IU and its Cartesian coordinate.

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