UV-Induced Amino $\rightarrow$ Imino Hydrogen-Atom Transfer in 1-Methylcytosine

Igor Reva,‡ Maciej J. Nowak,*‡ Leszek Lapinski,† and Rui Fausto‡

†Institute of Physics, Polish Academy of Sciences, Al. Lotnikow 32/46, 02-668 Warsaw, Poland
‡Department of Chemistry, University of Coimbra, 3004-535 Coimbra, Portugal

ABSTRACT: Monomers of 1-methylcytosine, isolated in low-temperature argon matrices, were excited with narrowband tunable UV light. Irradiation at $\lambda = 314$ nm resulted in syn$\rightarrow$anti photoisomerizations between the two minor imino-oxo forms of the compound, while the dominating amino-oxo form stayed intact. Subsequent irradiations at 308 nm (as well as at shorter wavelengths) led to the amino $\rightarrow$ imino hydrogen-atom transfer converting the biologically relevant amino-oxo tautomer into the imino-oxo forms. This is the first report on the amino $\rightarrow$ imino phototautomerism in 1-methylcytosine. The observed UV-induced syn$\rightarrow$anti photoisomerizations within the imino-oxo forms of 1-methylcytosine were found to lead to photostationary states. The photostationary [syn]/[anti] population ratio depended on the wavelength of the exciting UV light. This dependence was not monotonous. Spectral indications of the open-ring isocyanate product, generated from the amino-oxo tautomer upon UV (at wavelengths of 308 nm) irradiation, were also observed.

1. INTRODUCTION

Reports on the very fast decay of cytosine (Cyt) excited states have a substantial impact on the common opinion about the photostability of this nucleic acid base.1 As far as cytosine molecules isolated in supersonic jets are concerned, the recently published works reveal a very rapid excited state decay with a main component characterized by 1.1,2 1.9,3 and 1.2 ps4 lifetime. The last value was obtained following excitation of the molecule at 300 nm. For 1-methylcytosine (1mCyt), the decay time, measured after excitation at 300 nm, is very similar, 1.3 ps.4 It is believed that in these experiments the biologically relevant amino-oxo tautomer of both Cyt and 1mCyt was selected by excitation at 300 nm. These observations suggest that, in the amino-oxo forms of Cyt and 1mCyt, there are very effective deactivation pathways, allowing extremely fast (∼1 ps) depopulation of low-energy excited states.

Several theoretical computations of excited-state potential energy surfaces5–9 have been performed in search for the mechanisms of the ultrafast energy dissipation in electronically excited Cyt. In none of these studies, any possibility of UV-induced hydrogen-atom detachment from the NH$_2$ group was considered. However, this process (hydrogen-atom detachment) may play an important role in the photochemistry of Cyt and 1mCyt. Both compounds bear an amino group attached to a six-membered ring. Hydrogen-atom dissociation from the NH$_2$ group of electronically excited aniline was recently observed by King et al.10 This detachment occurred on the potential-energy surface of a repulsive πσ* state.10–12 Moreover, UV-induced amino $\rightarrow$ imino hydrogen-atom-transfer processes were observed by Akai et al.13–15 for matrix-isolated 2-aminopyridine and its derivatives. Most probably, the PIDA (PhotoInduced Detachment Attachment) mechanism,16 involving a key role of πσ* states, governs these photoinduced amino $\rightarrow$ imino hydrogen-atom transfer processes.

Very recently, an analogous phototautomeric process converting the amino-oxo form into the imino-oxo tautomer was observed for Cyt isolated in Ar matrices and UV-irradiated at wavelengths of 311–300 nm.17 Although this reaction was clearly observed, it was not very much pronounced. As a result of the amino $\rightarrow$ imino phototransformation, the amount of the imino-oxo tautomer of Cyt (initially constituting only a few percent of the total Cyt population) grew only by 1/3. One of the reasons for that was the comparatively low population of the amino-oxo reactant, since the dominating tautomer of Cyt is the amino-hydroxy form. Furthermore, upon UV excitation (at wavelengths of 311–300 nm), the amino-oxo tautomer of Cyt converted not only into the imino-oxo form but also into the amino-hydroxy form, in a competing oxo $\rightarrow$ hydroxy phototautomerism.

In the current work, we investigated the UV-induced transformations of monomeric 1mCyt isolated in low-temperature Ar matrices. In this compound, because of methylation at N1, the amino-hydroxy tautomer is not possible and the biologically relevant amino-oxo form strongly dominates. Hence, the amino $\rightarrow$ imino phototransformation in 1mCyt could be anticipated to be substantially more pronounced than...
in the unsubstituted cytosine molecule. Application of a tunable source of narrowband UV radiation allowed a very detailed study of the dependence of the photoinduced transformations in 1mCyt on the wavelength of the exciting light.

2. EXPERIMENTAL SECTION

The sample of 1-methylcytosine (purity 99%) used in the present study was a commercial product supplied by Sigma. In order to prepare Ar matrices containing isolated monomers of 1mCyt, the crystals of the compound were heated to ca. 470 K in a miniature glass oven placed in the vacuum chamber of a helium-cooled cryostat (APD Cryogenics closed-cycle helium refrigeration system with a DE-202A expander). Low-temperature matrices were formed by codeposition of 1-methylcytosine vapors and argon onto a CsI window cooled to 15 K. The argon matrix gas was of spectral purity (N6.0), as supplied by Air Liquide. The IR spectra were recorded in the 4000–400 cm⁻¹ range, with 0.5 cm⁻¹ resolution, using a Thermo Nicolet 6700 FTIR spectrometer equipped with a KBr beam splitter and DTGS detector. Matrices were irradiated with the frequency doubled signal beam of the Quanta-Ray MOPO-SL pulsed (10 ns) optical parametric oscillator (fwhm ~0.2 cm⁻¹, repetition rate 10 Hz, pulse energy ~1.0 mJ) pumped with a pulsed Nd:YAG laser. Typically, at any chosen wavelength, the matrix was irradiated for 20 min.

3. COMPUTATIONAL SECTION

The geometries of the three lowest-energy isomeric forms of 1mCyt (see the structures presented in Scheme 1) were fully optimized using the density functional method DFT(B3LYP) with the Becke’s three-parameter exchange functional¹⁸ and the Lee, Yang, Parr correlation functional.¹⁸ The 6-31++G(d,p) basis set was applied in these calculations. At the optimized geometries, the harmonic vibrational frequencies and IR intensities were calculated at the same DFT(B3LYP)/6-31++G(d,p) level. The computed harmonic vibrational wavenumbers were scaled by 0.98 below 2000 cm⁻¹ and by 0.95 above 3000 cm⁻¹. All the calculations were performed with the Gaussian 03 program.²⁰

4. RESULTS AND DISCUSSION

There are five low-energy isomers of unsubstituted cytosine (Cyt).¹⁷,¹¹,²² For 1-methylcytosine (1mCyt), because of methylation at N1, there are only three low-energy isomeric forms (see Scheme 1). According to the results of calculations carried out at the MP2 and CCSD(T) levels,²³ the amino-oxo (AO) tautomer of 1mCyt should be the most stable, whereas the imino-oxo forms should be higher in energy: IO1 by 10.8 (MP2), 6.9 [CCSD(T)] and IO2 by 17.3 (MP2), 13.1 [CCSD(T)] kJ mol⁻¹.

4.1. UV-Induced Isomerizations Affecting Imino-Oxo Forms. In the current work, the monomers of 1mCyt were trapped from the gas phase into low-temperature Ar matrices. The IR spectrum of a freshly deposited matrix is presented in Figure 1 and in Figures S1 and S2 (Supporting Information).
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4.2. UV-Induced Isomerizations Affecting Amino-Oxo and Imino-Oxo Forms. The first indications of a decrease of the population of the AO form were observed after irradiation of 1mCyt with monochromatic UV (λ = 309 nm) light. More vigorous consumption of AO reagent occurred upon irradiation at 308 nm and at shorter wavelengths. This photoreaction was very efficient. The UV-induced decrease (by 70%) of the initial AO population (see Figure 4 and Figure S3 in the Supporting Information) was accompanied by the substantial increase of the population of the imino-oxo form IO2. This increase exceeded many times the amount of IO2 that might result from additional IO1 → IO2 transformation occurring upon irradiation at λ = 308 nm. Therefore, IO2 must be generated at the cost of the decreasing population of the AO tautomer (see Figures 2 and 3). The assignment of the IO2 structure to the species being photoproduced upon exposure of the matrix to UV light (at λ = 308 nm or λ = 304 nm) can be confirmed by comparison of the experimental spectrum growing upon irradiation with the theoretical spectrum calculated for IO2. Good agreement between the experimental and theoretical spectra, shown in Figure S2 in the Supporting Information (traces d and e), strongly supports the identification of IO2 as the product of the photoreaction.

It is possible that the primary product of the amino → imino phototautomeric reaction in 1mCyt is the IO1 form (Scheme 1). From this primary IO1 product, IO2 can be generated via the syn→anti isomerization. The experiments, carried out within the present work (see Figure S4, Supporting Information), have demonstrated that the conversion of IO1 into IO2 occurs upon UV irradiation at 308 nm.

The photostationary population ratio of the IO1 and IO2 imino-oxo forms was found to be a function of the UV wavelength used for excitation. In the 314–304 nm range, this dependence was monotonous. The shorter the UV wavelength was, the higher the population of IO2, relative to the population of IO1. Irradiations at 308 or 304 nm led to photostationary states with the [IO1]/[IO2] population ratio strongly shifted in favor of the IO2 isomer (Figure 5). A direct proof of the photoreversibility of the IO1 ↔ IO2 isomerization was obtained as a result of further irradiations of matrices with a high relative population of IO2. When UV λ = 314 nm light was used for such an irradiation (Figure 5A), the population of the IO2 form decreased, whereas the population of IO1 increased. This clearly shows that excitation with UV light can induce not only the IO1 → IO2 process but also the isomerization in the opposite IO2 → IO1 direction.

Upon the final irradiation at 314 nm, only the populations of IO1 and IO2 changed. That is why the comparison of the bands that increased and decreased during such irradiation with the spectra theoretically calculated for the two imino-oxo forms may serve as a basis for identification of IO1 and IO2. The good agreement between the experimental spectra of increasing and decreasing bands and the spectra calculated for IO1 and IO2, respectively, allowed a reliable assignment of both spectra to the structures of their carriers (Figure 6).

The results of the experiments described above clearly show that, for λ > 304 nm, the relation between the excitation wavelength and the photostationary [IO1]/[IO2] population ratio is monotonous. However, this relation is no longer monotonous for λ < 304 nm (see Figure S5B). Contrary to the previous irradiations at 314 and 304 nm, the irradiation at 280 nm resulted in a decrease of the population of IO2 and the corresponding increase of the IO1 population.

Figure 2. Fragment of the infrared spectrum of 1-methylcytosine monomers isolated in an Ar matrix recorded: (black) after deposition of the matrix; (green) after irradiation at λ = 314 nm; (blue) after subsequent irradiation at λ = 310 nm; (red) after subsequent irradiation at λ = 308 nm. In the 1085–1065 cm⁻¹ region, the traces black, green, and blue overlap, showing that the AO form was not consumed upon irradiations at λ ≥ 310 nm. Upon irradiations at 314 and 310 nm, the population of IO2 grew at the cost of the IO1 isomer. Comparison of this very moderate growth with the sizable increase occurring upon irradiation at 308 nm (consuming the AO form) is a striking evidence of the amino → imino phototautomeric process in 1mCyt.

Figure 3. Fragments of the infrared spectrum of 1-methylcytosine monomers isolated in an Ar matrix recorded: (black) after deposition of the matrix; (blue) after irradiation of the matrix with UV (λ = 314 nm) laser light; (red) after subsequent irradiation at λ = 308 nm. The intensities of the bands marked as AO are the same in the spectra recorded after deposition and after irradiation at λ = 314 nm (traces black and blue).

corresponds to consumption of approximately 45% of the initial IO1 population.
Comparison of the spectra recorded after the final irradiations at 314 and 280 nm (top traces in parts A and B of Figure 5) with the spectra recorded after the first irradiation at 314 nm (second traces from the bottom in parts A and B of Figure 5) clearly shows that populations of both IO1 and IO2 imino-oxo forms increased (upon UV irradiations at 308 or 304 nm) at the cost of the decreasing population of AO. This is a further and unequivocal proof of the amino $\rightarrow$ imino phototransformation occurring in 1mCyt (Scheme 1).

4.3. Populations of 1-Methylcytosine Isomers. Photoreactions observed for matrix-isolated 1mCyt allowed assignment of the bands observed in the IR spectra of the compound to its AO, IO1, and IO2 forms. This assignment is presented in detail in Figure 4 and in Figures S1 and S3 in the Supporting Information. It served as a basis for estimation of relative populations of AO and IO1 isomers $[AO]/[IO1]$ in an Ar matrix before any irradiation. The relative occupancy $[AO]/[IO1]$ was assessed as a ratio of experimentally determined integrated intensities ($I_{exp}$) of the bands due to AO and IO1, scaled by the calculated absolute intensities ($A_{theor}$) of the corresponding theoretical bands (see comment in the reference section26):

$$\frac{[AO]}{[IO1]} = \frac{m \sum_{i=1}^{m} I(AO)_{iexp} / A(AO)_{theor}}{n \sum_{j=1}^{n} I(IO1)_{jexp} / A(IO1)_{theor}} = \frac{100}{(9 \pm 4)}$$

(1)

Regarding the population of the IO2 isomer, it can be noted here that its amount in the initially deposited sample is very low, and only six strongest bands can be distinguished (see Figures S1 and S3, Supporting Information). These bands are overlapped with absorptions of the other isomers and/or blend into baseline, which precludes a reliable assessment of their experimental intensities. Only a very rough estimate could be made, showing that the $[IO2]/[IO1]$ ratio is approximately 0.17.

It is interesting to compare the experimental relative populations assessed in the present experiment with the theoretically calculated stabilities of 1mCyt isomers. Haránczyk et al. calculated the MP2 thermal free energies of AO, IO1, and IO2 to relate as 0.0, 9.3, and 15.4 kJ mol$^{-1}$23 Taking these
relative energies, the population ratio \([\text{AO}]/[\text{IO1}]/[\text{IO2}]\) should be 100/9.3/1.9 assuming a thermal equilibrium at the sublimation temperature of 470 K. This computational estimate agrees nicely with the experimental assessment of populations obtained in the present experiments.

4.4. UV-Induced Ring-Opening Isomerization of 1-Methylcytosine. The phototransformations observed for matrix-isolated 1mCyt are not restricted to isomerizations converting the \(\text{AO}, \text{IO1}, \text{and IO2}\) forms into each other. The striking evidence of this is the new, structured band at 2261 cm\(^{-1}\) (see Figure 7 and Figure S5, Supporting Information).

that appears in the IR spectra of UV-irradiated 1mCyt. The carrier of this new band must be generated by phototransformation of the \(\text{AO}\) tautomer. The species characterized by the band at 2261 cm\(^{-1}\) is not produced upon irradiation at 310 nm (nor at longer UV waves), but it starts to be generated upon irradiation at 309–308 nm, which causes consumption of the \(\text{AO}\) form. Observation of photoproducts characterized by the structured bands at 2266 and 2262 cm\(^{-1}\) was previously reported for Cyt\(^{17}\) and for 1-methyl-2(1H)-pyrimidinone\(^{27}\) (Table 1). The frequency and the structured profile of these bands are characteristic of the IR absorptions due to the “antisymmetric” stretching vibrations of the \(-\text{N}==\text{C}==\text{O}\) isocyanate group.\(^{17,27,28}\) In a detailed study, carried out for 1-methyl-2(1H)-pyrimidinone,\(^{27}\) the open-ring conjugated isocyanate structure of the photoproduct absorbing at 2262 cm\(^{-1}\) was unequivocally confirmed. By analogy, it seems very probable that conjugated isocyanates are also photoproduced from the \(\text{AO}\) forms of Cyt and 1mCyt. Though the structures of 1-methyl-2(1H)-pyrimidinone and the \(\text{AO}\) form of 1mCyt are quite similar, they differ by the presence of an extra amino group in 1mCyt. The presence of this extra NH\(_2\) group in 1mCyt makes it possible for the corresponding open-ring conjugated isocyanate to exist in isomeric structures with intramolecular hydrogen bonding (see Figure 7 and Tables S1 and S2 in the Supporting Information).

The opening of the pyrimidine ring, leading to transformation of the \(\text{AO}\) forms of Cyt and 1mCyt into conjugated isocyanates, should occur by the classical photochemical process known as \(\alpha\)-bond cleavage or Norrish type I photoreaction.\(^{29}\) Appearance of open-ring conjugated ketenes, photogenerated in analogous \(\alpha\)-bond cleavage processes, was observed for a number of heterocyclic compounds, such as 4(3H)-pyrimidinone,\(^{30}\) 2(1H)-pyridinone,\(^{31}\) 3(2H)-pyridazine-none,\(^{32}\) and \(\alpha\)-pyrone.\(^{33}\) Therefore, ring-opening in Cyt and 1mCyt, leading to conjugated isocyanates, would be just another example of the photochemical behavior typical of the whole class of heterocyclic compounds (Table 1).

5. CONCLUSIONS AND IMPLICATIONS

Monomers of 1-methylcytosine were isolated in cryogenic argon matrices. In a newly formed matrix, ca. 90% of 1mCyt molecules adopt the amino-oxo form \(\text{AO}\). The remaining population adopts the imino-oxo form, where the \(\text{IO1}\) isomer dominates, while the \(\text{IO2}\) form is barely discernible. The UV-induced hydrogen-atom-transfer process, converting the \(\text{AO}\) form into the imino-oxo \(\text{IO1}\) and \(\text{IO2}\) isomers (Scheme 1), was observed for the matrix-isolated compound. This was the first observation of the amino \(\rightarrow\) imino phototautomerism in 1mCyt. As much as 70% of the initial population of \(\text{AO}\) was consumed in this photoreaction, and the vast majority of it was transformed into the imino-oxo forms. Hence, the scale of the photoduced amino \(\rightarrow\) imino hydrogen-atom-transfer reaction occurring in matrix isolated 1mCyt was large (Figure 4).
Considering the mechanism of the \( AO \rightarrow (IO_1 + IO_2) \) photoreaction, the hydrogen-atom detachment from the NH\(_2\) group should play a key role. Such a hydrogen-atom detachment, occurring on the surface of the repulsive \( \pi\sigma^* \) state, has recently been observed for gaseous aniline by TKER spectroscopy.\(^{10}\) It is likely that the present work will stimulate new TKER experiments on the photodissociation of hydrogen atoms from the NH\(_2\) group of Cyt and 1mCyt in supersonic jets.

Alongside the amino \( \rightarrow \) imino phototautomeric transformation, a concomitant \( IO_1 \leftrightarrow IO_2 \) syn–anti isomerization was observed for matrix-isolated 1mCyt. Investigation on this syn–anti photoisomerization revealed that the process is photoreversible and leads to a photostationary state. Using monochromatic excitation light, it was possible to study the dependence of the photostationary \([IO_1]/[IO_2]\) population ratio on the wavelength of UV light used for irradiation. The photostationary \([IO_1]/[IO_2]\) population ratio was found to be a nonmonotonous function of the wavelength of the applied UV radiation.

Spectral indication of another channel of phototransformation of the AO form of 1mCyt was also found. The

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\(^{10}\)Characteristic IR bands at ca. 2260 and 2140 cm\(^{-1}\) were observed as spectral indications of conjugated isocyanates and ketenes, respectively.

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Table 1. Ring-Opening by \( \alpha \)-Bond Cleavage Observed in a Series of Heterocyclic Compounds Isolated in Low-Temperature Argon Matrixes\(^{10}\)
The observed photoproceses transforming the biologically relevant AO form of 1mCyt occurred upon excitations with UV light of $\lambda \leq 309$ nm. This may be related to the results of the study of the excited-state dynamics of Cyt and 1mCyt using femtosecond pump–probe photoionization mass spectrometry. The obtained decay time was about 1.3 ps at 300 nm excitation, but when the excitation wavelength was tuned to 310 nm, the decay suddenly became much slower with a time constant of about 3.2 ps. The decay following the excitation at 310 nm had also a much longer-lived component. The shorter ($\sim 1$ ps or subpicosecond) decay time, for excitations at $\lambda < 310$ nm, may be related to the fast photoisomerizations. The hydrogen-atom detachment from the NH$_2$ group, currently observed for 1mCyt and Cyt, may be considered as one of the candidates for such a fast process.

The origin of the UV absorption band in the AO form of 1mCyt in a supersonic expansion was found at 313.4 nm. The fact that the phototransformations (as well as the shortening of the excited state lifetime) occur at wavelengths $\leq 309$ nm, hence close to the origin of the absorption band, suggests that the barriers for the processes consuming the AO form are low.

Contrary to the common belief that short excited-state lifetimes, observed for nucleic acid bases, implies their high stability against photoinduced structural changes, our experiments demonstrated that fast deactivation of the excited states does not hinder photoisomerizations in monomeric 1mCyt molecules. The results obtained in the current work provide a new insight into the photochemistry and photophysics of 1mCyt.

The present study should stimulate theoretical calculations on low-energy dissociative $\pi\pi^*$ states in Cyt and 1mCyt. So far, such excited states of repulsive character were totally neglected in the computational investigations of the photophysics of cytosines. The same concerns the photoinduced cleavage of the $\alpha$-bond, with respect to the C$=$O group in cytosine. Although the Norrish type I ($\alpha$-bond cleavage) reaction is known to occur in a wide variety of carbonyl compounds, it was never considered in relation to the biologically important AO forms of cytosines.

### ASSOCIATED CONTENT

#### Supporting Information

Figures S1, S2, and S3 showing infrared spectra of 1-methylcytosine isolated in an Ar matrix recorded before and after irradiation at 308 nm. Figure S4 where the photoreversible 1O1 $\rightarrow$ 1O2 isomerization as well as the phototautomeric amino $\rightarrow$ imino conversion are shown. Figure S5 presenting the structured band in the range 2300–2200 cm$^{-1}$ growing upon UV irradiations. Table S1 containing the relative energies and optimized geometries of different isomers of the open-ring conjugated isocyanate photoproduct. Table S2 collecting the calculated IR spectra of different isomers of the open-ring conjugated isocyanate. This material is available free of charge via the Internet at http://pubs.acs.org.

### AUTHOR INFORMATION

Corresponding Author
*E-mail: mnjnowak@ifpan.edu.pl.

### REFERENCES

In this procedure, six bands due to the AO form ($n = 6$, the bands at 3557, 3437, 1593, 1490/1487, 1369, 1144 cm$^{-1}$) and four bands due to the IO1 form ($m = 4$, the bands at 1456, 1324, 1115, 813 cm$^{-1}$) were taken into account. These bands were well separated from the others in the spectrum and were credibly assigned to the given theoretically calculated normal mode.