Photodissociation Dynamics of DNA Bases

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Abstract. In order to access the damaging mechanisms of DNA and its bases on the molecular level by T Hz radiation, it is necessary to create a database of assessing damage at different spectral regions. Possible damage by T Hz radiation will be assessed by comparison. Furthermore, the expose of the cytosine to radiation at 157 nm, suggest that photochemical bond breaking is the only damaging mechanism in the vacuum ultraviolet region of the spectrum, (157–170) nm where one photon breaks one chemical bond.

Key words: 157 nm, Cytosine, DNA photo-damage, mass spectroscopy, molecular dynamics, photo ablation, T Hz radiation, vacuum ultraviolet

1. Introduction

The lack of any spectroscopic data of biological materials in the T Hz region of the spectrum together with the lack of convincing theories to describe the interaction of radiation with matter prevents a thorough understanding of damaging mechanisms of biological matter in this spectral region. Damage of biological material is commonly refer as breaking or changing chemical bonds which retain the biological functionality and integrity of the living specimens. It is obvious therefore that DNA damage in the T Hz region is the first step towards advancing the state of the art in this direction. Possible damaging mechanisms in the T Hz region can be assessed and been understood by comparison to well defined damage in other spectral regions.

In this work by applying the experimental techniques of laser mass spectroscopy, and laser ablation, it was found that structural damage of cytosine (one of the DNA bases), is accompanying illumination at 157 nm, and always the energy of one photon is compensated of breaking one chemical bond. Hence structural damage of DNA at 157 nm is a critical issue of creating a database over a wide spectral region, and thus assessing DNA damage in the T Hz region by comparison. This is more so compared to longer wavelengths such as 193 nm, due to the higher photon energy at 157 nm and the corresponding higher fragmentation probability

Figure 1. Experimental set-up for obtaining the mass spectrum of cytosine at 157 nm. LB: laser beam, L: lens, VC: vacuum chamber, TM: turbo molecular pump, W: LiF: window, S: sample, P: photofragments, QMS: quadrupole mass spectrometer.

in comparison to other wavelengths in the whole electromagnetic spectrum. Understanding and limiting structural damage at 157 nm is thus crucial for any further progress. Furthermore the expose of the cytosine to vacuum ultraviolet (VUV) radiation at 157 nm, revealed that photochemical bond breaking is the only bond breaking mechanism in the VUV region of the spectrum, (157–170) nm. In addition photochemical bond breaking via multi-photon absorption is taking place in the UV region of the spectrum up to 300 nm, while thermal heating is the dominant damaging process at longer wavelengths. By extrapolating these results to the THz region of the spectrum, it is expected molecular damage (if it is any), to be only thermal.

2. Experimental

The experimental approach for the study of the dissociation dynamics of the organic matter, (in our case the DNA base, cytosine) is based on mass spectroscopy following irradiation in various spectral regions from the deep ultra violet to the THz spectral region together with laser ablation studies. Under irradiation in the VUV, the parent molecule disintegrates to small photo-fragments, atomic, diatomic or triatomic. This it will be the fingerprint of bio-damage on the molecular scale.

For the mass spectroscopic studies, the experimental apparatus consists mainly of the 157 nm laser source and the vacuum chamber, where the quadrupole mass spectrometer and the cytosine crystal sample were placed, 1 mm apart from the quadrupole mass spectrometer at right angle to its axis, Figure 1. The all stainless steel 316 vacuum chamber was evacuated at 10^{-6} mbar using a turbomolecular pump. The laser source used for this experiment was of the fast discharge type, and

it had been described previously. The laser head delivered 1 ± 0.2 mJ at 157 nm per pulse at 10 Hz repetition rate, and the pulse duration was ~ 12 ns at FWHM. The laser beam was focused on the samples, using a LiF lens of 4 cm focal length. After the photodissociation of the parent molecule, the molecular photofragments were ionized using an electron gun inside an isopotential chamber (Wehnelt). The molecular ions were focused with an Eizen lens, and eventually they were directed alongside the quadrupole mass filter. The upper detection limit of this filter was 300 amu. The ions after entering the mass filter were deflected at right angle, and they were detected using a high gain $\sim 10^8$, secondary electron multiplier (SEM).

3. Results and Discussion

3.1. PHOTO-DAMAGING MECHANISMS

For the understanding of the mechanisms of interaction of laser light with DNA, the complete microscopic description of the photo dissociation dynamics involved in the scission of the DNA crystals, and the dynamics of the extraction of the photoproducts is required.

Up to now several models have been developed describing the interaction of organic matter with light in alongside with the improvement of the experimental methods. However, despite the active practical use of lasers, and the extensive experimental and theoretical studies in this field, the description of the underlying elementary processes of photo dissociation dynamics is rather poor and the optimum values of the experimental parameters are mostly obtained by trial and error. The analytical models which describe the laser ablative process are based on different assumptions, such as the thermo-fluctuation sublimation of molecules from the surface (surface vaporisation), explosive bulk adsorption due to a non-equilibrium phase transition or critical pressure gradient, and hydro dynamical models [1–3]. Up to now various theories, which analyse the role of the excited species in ultraviolet laser materials ablation, have been developed. On the other hand, the complexity and the diversity of the processes which are involved in the laser ablation dynamics, such as laser excitation of the absorbing molecules/bonds and energy transfer from the excited parent molecules into the internal and the translation degrees of freedom of the photo fragments, require for their description the analytical expression of the potential energy surfaces of the parent molecules and the photo fragments, for both the ground and the excited states.

In the case of illumination of cytosine with light, mainly two kind of processes are induced on the molecules: the first being excitation followed by relaxation to the ground state, and the second being excitation followed by molecular disintegration, Figure 2.

Regarding the first process, which exclusively accompanies illumination at longer wavelengths; the electronic excitations of the molecular specimens could relax by internal conversion to the vibration excitations of the molecule. Redistribution of the intermolecular energy (due to the different geometry of the molecule in the

Figure 2. Generalized photodissociation model of organic molecules following illumination with deep-UV laser (long arrow). In this case molecular disintegration is taking place. Illumination at longer wavelengths (short-arrow) is usually accompanied by photon emission and heating.

ground and the excited states) that initially appears in a particular vibration mode of an excited molecule has been found to be fast ~ 1 ps. A much slower process of cooling the vibration hot molecule follows the intermolecular energy redistribution. A computer simulation of vibration cooling reveals the characteristic time of few tens of pico-seconds, and this value is consistent with experiments. Cytosine in DNA is connected to guanine with hydrogen bonding having a small amount of activation energy of few tens of an electron volt. Therefore the rise of the bulk temperature in DNA crystals as the laser pulse at longer wavelengths heats it, could have the effect of breaking the hydrogen bonds. Alternatively, deep UV excitation ('long arrow' in Figure 2) is followed by monomer dissociation. In this case, the laser energy is expended in breaking the chemical bonds, and forming molecules with smaller number of atoms. The excess energy is converted to translation energy of the photofragments. The energy transfer in this case is considerably faster than the vibration relaxation mentioned previously and a molecular dynamics model attempting to explain the basic ablative processes at a molecular level, should take into account the two broad categories of the mechanisms involved in the process of vibration excitation and photo-fragmentation.

3.2. LASER ABLATION

VUV and UV laser mass spectroscopy together with absorption spectroscopy are the main experimental tools for investigating photodissociation dynamics of organic molecules at different wavelengths. However absorption spectroscopy requires the development of homogeneous thin films on suitable substrates in order to give reliable results. Taking into consideration that cytosine thin films are produced in crystalline form from liquid melts at moderate temperatures and in the presence

Figure 3. Scanning electron image of thin cytosine film deposited on silica substrate. The film was prepared by dipping the substrate at liquid melt in Ar background pressure. Despite the fact that the film appears to be uniform, it is polycrystalline.

of argon buffer gas, well-controlled conditions are required for film homogeneity, Figures 3–4. In the case of non-uniform polycrystalline films the absorption coefficient can be measured using the laser ablative method [4], which provides reliable data regarding the value of the absorption coefficient and the laser ablative threshold [5].

The ablative fluence threshold, which is observed in all etch rate measurements, separates the two mechanisms responsible for the ejection of the photofragments, i.e. surface vaporization at low laser fluence and collective ejection of the molecular photofragments from photodissociation. This dynamic model suggests that the ablation process being characterized by a photon flux threshold Π_0 (in photons/cm³·sec), which defines the concentration of photons absorbed per unit time required to overcome various relaxation paths.

Similarly Sutcliffe et al. [6] in their dynamic model suggested that the photon flux threshold is independent of the laser wavelength and depends only on the material. This characteristic parameter in laser ablation is calculated in the present work and the results are compared to those of the dynamic model. In Sutcliffe's

Figure 4. Scanning electron image at a different surface point of the sample. The film is not uniform distributed over the dipped substrate.

work of the dynamic ablation model, the set of the parameters Π_0 and n_0 play the key role in the ablative process. The flux threshold I_T is defined by the equation:

$$I_T = \Pi_0 \frac{(hc)}{(\alpha\lambda)}$$

Where α is the absorption coefficient, λ is the wavelength, I_T is the corresponding laser intensity threshold, and $I_T = F_T/t_p$, F_T been the laser energy threshold and t_p the pulse duration. The duration of the laser pulse t_p is measured at FWHM.

The definition of I_T of the ablative process is consistent with the usual definition of the light intensity, but it might introduce an error to the calculations since it assumes that the energy is deposited after the laser pulse has entered the sample. This discrepancy is attributed partially to Beer's law, which assumes that the ablative process occurs after all the laser pulse has traveled through the sample and also, that the interaction of the laser light with the sample and the ablative process are taking place in a different time scale. However, photoacoustic detection of the stress pulse generated by the ejected material shows that ablation of the material (mainly photodissociation) occurs within the time scale of the laser pulse [7]. Moreover, simulations of the laser ablation based on the solutions of the classical equations of motion, predict the onset of the ablative process a few picoseconds after the bondbreaking step [8, 9].

Figure 5. Etch rate of cytosine versus laser fluence at 157 nm. The threshold fluence was estimated to be 1 mJ/cm^2 and the absorption coefficient to $7 \mu\text{m}^{-1}$.

The etch depth per pulse, as a function of the incident laser fluence for cytosine is shown in Figure 5 for 157 nm. The dispersion of the experimental data at high fluences is due to the fluctuations of the energy of the laser beam. As it is generally accepted [10], the etch depth per pulse, d , is given by the Beer's law

$$d = \alpha^{-1} * \ln(F/F_T)$$

Where α , is the absorption coefficient in cm^{-1} of the material at a given wavelength, F is the fluence of the laser pulse and F_T is the threshold fluence which describes the minimum fluence of light where the photodissociation process starts to be competitive to the thermal relaxation processes, such as intersystem crossing or collision quenching. These processes merely degrade the energy without engendering any photodissociation [6]. The least square fitting of the Beer's law to the experimental results, suggests for the threshold fluence F_T , and the absorption coefficient α at 157 nm the values of $F_T = 2 \text{ mJ/cm}^2$ and $\alpha = 7 \times 10^4 \text{ cm}^{-1}$ respectively, Figure 5.

Taking into consideration the experimental conditions at 157 nm, the average photon flux near threshold F_0 is: $F_0 = 4 \times 10^{25} \text{ photons/cm}^2 \cdot \text{sec}$. Multiplication of Π_0 by t_p gives the photon flux ρ_T , which is a parameter independent of time, $n_0 = 6 \times 10^{17} \text{ photons/cm}^3$. The parameter ρ_T defines the number of photons per unit volume of the sample needed to initiate ablative etching.

An additional parameter accounting for the proper description of the ablating process, is the number of photons per molecule at the threshold e_T , $P_T = n_0 * V_{\text{mol}}$, where V_{mol} stands for the molecular volume, $V_{\text{mol}} = N_A^{-1} * m_{\text{mole}}/\bar{n}$, N_A is the Avogadro number, m_{mole} is the molecular weight of the cytosine, and n_0

Figure 6. Mass spectrum of cytosine at 157 nm with 0.1 mJ of laser energy.

is the sample's density. On the basis of the calculations, the number of photons per molecule to initiate the ablation process is: $P_T \sim 10^{-4}$ photon/molecule. This number is rather indicative of the low energy fluence which is required to initiate the ablative process, and is in agreement with our experimental results that photodissociation products could be observed even at low laser energies at 157 nm.

3.3. 157 NM MASS SPECTROSCOPY

Mass spectroscopic results suggest that at 157 nm photochemical bond breaking is taking place due to the fact that the density of the dissociative excited electronic states of the small diatomic or triatomic radicals which are correlated with the excited electronic states of the monomer of the different polymeric materials at 7.8 eV is large, therefore photodissociation at this wavelength is the only damaging mechanism. The molecular photofragments are flying apart with supersonic speed, and the presence of steep dissociative excited electronic states is inherent to the molecular structure. For higher photon flux only photofragments of small masses were observed suggesting sub sequential bond breaking.

The laser energy was kept as low as possible in order to produce significant etching of the sample and therefore good signal to noise ratio.

For comparison, the background mass spectrum was recorded with the laser off. The mass spectrum in this case indicates that there is not any significant degassing at the background pressure of 10^{-6} mbar. The mass spectrum under laser irradiation up to 0.8 mJ/cm^2 per pulse was recorded and the total number of pulses delivered on the sample was 1000 at 10 Hz repetition rate of the fluorine laser.

The photo fragments at 157 nm are the following: H, H₂, C, CH, CH₂, N, O, OH, H₂O, C₂H, C₂H₂, CN, C₂H₃, HCN, N₂, CO, C₂H₄, N₂H, NO, C₂H₆, H₂CO, N₂H₂, CH₂NH, Figure 6. Photo fragments with mass higher than 30 m/e have a lower probability for dissociation than the amide group. This is due to the presence of a repulsive excited state of the amide group for energies higher than 6.3 eV. The molecular photo fragments are the products of either direct dissociation of the parent molecule or recombination of the various excited fragments. For example the photo fragment at 29 amu is not directly linked to the parent molecule. This radical is created through recombination of the photo fragment C₂H₄ with one free hydrogen atom. Considering that the high molecular weight of the monomers; we come to the conclusion that there is a complete bond breaking of the polymeric chain under irradiation at 157 nm.

The main photo fragments from the dissociation are the following: C₂H₂, CN, C₂H₃, HCN, C₂H₄. The most predominant peak corresponds to 26 amu, and this peak can be assigned to the C₂H₂ or the CN radicals. The C₂H₄ radical can be formed in two different pathways. One pathway is by breaking the C–C and C=C bonds of the parent molecule, and the other one is by breaking one C–C and one C–N bond. Considering that the photon energy at 157 nm is 7.8 eV; two photons at 157 nm are required to form the C₂H₄ radical in both cases. Take into account that the excess energy is converted to kinetic energy of the photo fragments; the speed of the ejected photo fragments is expected to be of the order of 10³ m/sec.

4. Conclusions

Photochemical damage of cytosine is taking place at 157 nm. One photon at this wavelength is enough to initiate photo-dissociation by breaking one chemical bond. This result justifies the use of 157 nm photons to ‘calibrate’ and hence to assess light induced damages at longer wavelengths. Small radicals and clusters are produced via atomic or molecular recombination. The photo dissociation of the amide group at 157 nm has a relatively higher probability to take place than the photo dissociation of the different groups within the molecule. The absorption coefficient of cytosine at deep UV wavelengths was measured to be of the order of 7 μm⁻¹. It is expected by extrapolation of the results that damage of DNA bases in the THz region will be only thermal due to the accumulation of the electromagnetic energy within the target volume.

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