Strand break formation in plasmid DNA irradiated by nanosecond XUV-laser pulses

E. Nováková[1], M. Davídková[2,3], L. Vyšín[1], T. Burian[1], M. E. Grisham[4], S. Heinbuch[4], J. J. Rocca[4], L. Juha[1]

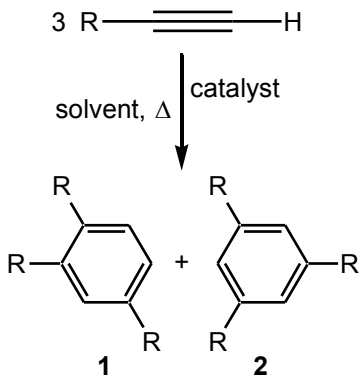
[(1)]Department of Radiation and Chemical Physics, Institute of Physics AS CR, Na Slovance 2, 182 21 Prague 8, Czech Republic

[(2)]Department of Radiation Dosimetry, Institute of Nuclear Physics AS CR, Na Truhlářce 39/64, 180 86 Prague 8, Czech Republic

[(3)]Faculty of Nuclear Sciences and Physical Engineering, Czech Technical University in Prague, Břehová 7, 115 19 Prague 1, Czech Republic

Abstract

The development of the desktop, repetitive XUV laser based on collisionally pumped transition of Ne-like Ar ions in a pinching capillary discharge [1] is of interest for numerous applications in radiation biophysics. Ionizing radiation induces a variety of DNA damages including single-strand breaks (SSBs), double-strand breaks (DSBs), abasic sites, modified sugar and bases. Most theoretical and experimental studies have been focused on DNA strand scissions, in particular production of DNA double-strand breaks. The complexity of lesions produced in DNA by ionizing radiations is thought to depend on the amount of energy deposited at the site of each lesion. We have studied the nature of DNA damage induced directly by the pulsed 46.9 nm radiation provided by a capillary-discharge Ne-like Ar laser (CDL). Different surface doses were delivered with a repetition rate of a few Hz and an average pulse energy ~ 1 μJ. A simple model DNA molecule, i.e., dried closed-circular plasmid DNA (pBR322), was irradiated. The agarose gel electrophoresis method was used for determination of both SSB and DSB yields.



Scheme 1

INTRODUCTION

The study of radiation damage to biomolecules is important for understanding of the mechanisms of radiation damage to cell, tissues, and living organisms. The primary target for radiation-induced cell damage is the DNA molecule [2].

Ultrasoft X-rays induce almost exclusively ionizing radiation damage. Direct ionization of binding electrons in the sugar or the phosphate or indirect pathways due to water radicals produced in the track lead to a formation of strand-breaks. Two SSBs on opposite strands have been assumed to lead to a DSB if separated by 10 bp or less. Double-strand breaks (DSBs) are considered the most critical DNA lesions induced by ionizing radiation. Damage to bases leads to a variety of base alterations. At the system of DNA irradiated under vacuum is predominantly involved direct effect of irradiation.

Experimental studies where samples of plasmid DNA were irradiated in vacuum with photons of energies in the 7-150 eV range provide evidence for the ability of photons as low as 7 eV to induce both SSB and DSB [3]. The primary ionizing radiation generates low energy secondary electrons in biological materials through photoelectric effect. These low electrons are expected to induce biological effects more effectively than higher energy electrons/photons [4]. They determined, using a monolayer DNA sample irradiated under vacuum conditions with extremely low-energy electrons (below 20 eV), that dissociative electron attachment play an important role in DNA strand breakage and in the decomposition of nucleobases.

The biological effects of low-energy X-rays were currently studied using single sub-nanosecond 1-keV X-ray pulse produced by a large-scale, double-stream gas puff target, illuminated by sub-kJ,

near-infrared (NIR) focused laser pulses [5]. The yields of SSBs and DSBs as well as the SSB/DSB ratio were in very good agreement with the results of other groups using soft X-ray tubes and synchrotron radiation, i.e., at much lower dose rates. The ability of the plasma source to induce measurable radiobiological change by an action of even a single shot was demonstrated.

MATERIALS AND METHODS

DNA sample preparation and measurement of strand breaks

The pBR322 DNA plasmid (4361 bp) was purchased from Fermentas Life Sciences (York, UK). More than 95% of the used DNA was characterized to be in the supercoiled form. To prepare thin films of DNA, we pipetted 5 µl of solution containing 110 ng of plasmid DNA in a Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA, pH = 7.6; abbreviated as 1 x TE buffer) onto a glass coverslip (Hirschmann Laborgeräte, Eberstadt, Germany), and allowed to dry in air. The DNA samples were prepared immediately before irradiation, and redissolved in 8 µl of 1 x TE buffer just after irradiation. After one dehydration-rehydration cycle, the supercoiled decreased to about 92%.

After drying, a film of DNA/buffer solutes having a diameter of 3.5 mm was formed on the coverslip. The thickness of DNA samples was measured by a surface profiler (Alpha Step 500, Tencor Instruments, Mountain View, CA) to be 65-70 nm. The fraction of XUV pulse energy deposited in the sample was estimated from the sample thickness, the density of DNA (1.7 g/cm^3) [6] and the elemental composition of pBR322 DNA ($C_{6.8}H_{9.8}N_{3.2}O_{2.4}P_{0.9}$) using the X-ray atomic absorption cross-section tables [7]. It has been found that for our sample and radiation ~ 99.2% of the energy is deposited in the irradiated material.

The samples were analyzed to evaluate the fractions of SSBs and DSBs by agarose gel electrophoresis using established method [5]. Irradiated and control samples containing about 110 ng DNA were mixed with 2 µl of 30% (w/v) glycerol/0.25% (w/v) xylene cyanol/0.25% (w/v) bromophenol blue. The mixtures were applied to a neutral 0.8% agarose gels and run in 0.5 x TAE buffer (20 mM Tris, 10mM sodium acetate, 1 mM EDTA, pH=8.0) at 100 V. Under these conditions, undamaged 'supercoiled' (form 1) DNA migrated faster than did a linear form (form 3) DNA, followed by a relaxed form (form 2) DNA. The gels were stained with SYBR Green I solution (1:10000, Sigma Aldrich, Taufkirchen, Germany). Images of the gels were taken on a UV transilluminator table (UVT-20ME; Herolab, Wiesloch, Germany) with an Olympus C-720 digital camera. Obtained images were transformed to black and white format and peaks corresponding to different forms of DNA were integrated by home made software Luthien.

XUV source

The experimental setup we used for the radiobiological experiments is shown in Fig. 1(a). The samples were irradiated with the beam of a Ne-like Ar capillary discharge laser operating at 46.86 nm wavelength on a 3p→3s transition (J=0 to 1) in Ne-like argon. Full details of this table-top soft X-ray laser have been given in the previous publications [4,8]. The discharge driven by a 22 kA peak current occurs through a 380 mTorr argon gas in a 21 cm long and 3.2 mm diameter capillary tube. Laser pulse energy, monitored by means of the vacuum photodiode, was adjusted to 1.2 µJ (3 x 10^{11} XUV photons/pulse) with a high shot-to-shot stability. Optimization of the plasma conditions in this device can yield up to 10-µJ pulses [12]. Measured pulse duration is of 1.5 ns FWHM (Fig. 1b).

Axial emission spectrum with one dominant 46.9 nm spectral line is shown in Fig. 2. The spectrum was obtained with flat-field XUV spectrometer equipped with a back illuminated X-ray charge coupled device (CCD; Princeton Instruments) behind a 0.40-µm aluminium foil.

The samples were placed into the vacuum chamber at a distance 105 cm from the source and irradiated at a repetition rate of 3 Hz. Typically, samples were

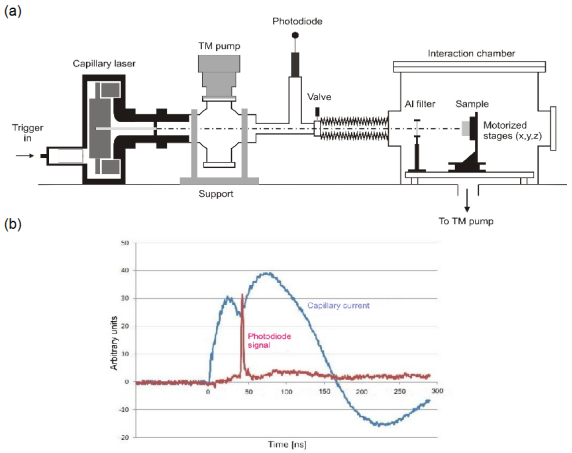


Fig. 1. (a) Scheme of the CDL and the vacuum chamber for irradiating plasmid DNA. (b) Time progress in discharge current and laser output intensity.

irradiated when the vacuum pressure had reduced to less than 10^{-5} mbar within the sample chamber. Note that control sample is subjected to the same vacuum cycle, but is not exposed to XUV light. The beam position is checked by installing a Ce:YAG scintillation crystal (Crytur Ltd., Czech Republic) at the sample position and viewing the fluorescence due to the incident radiation. The broad-band incoherent UV-Vis radiation emitted from the plasma column of the capillary discharge was filtered out using 0.15-µm and 0.4-µm thick aluminium foils (>17 eV, Goodfellow Cambridge Ltd, England).

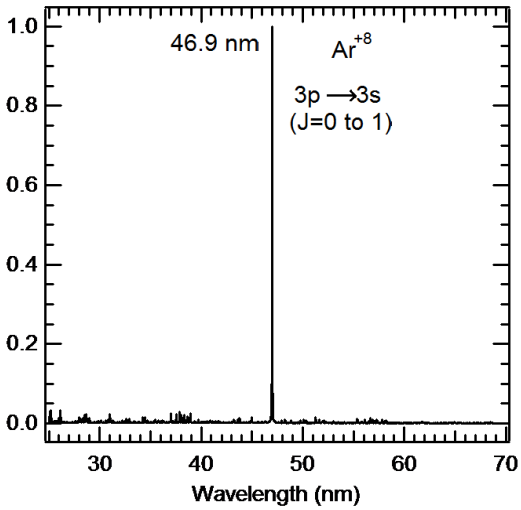


Fig. 2. XUV lasing at 46.9 nm on the 3p→3s, J=0 to 1 transition in Ne-line argon. Axial emission spectrum (normalized at 46.9 nm) in the region between 25 and 70 nm.

RESULTS AND DISCUSSION

The DNA molecule in solid films adopts a double-helix conformation known as A-form. Under physiological conditions, the dominant form of DNA is the B-form. In very low humidity, the B conformation changes to more compact, with 11 bases per turn instead of 10.5 in the B form. Its base pairs are tilted rather than perpendicular to the helix axis. The transition of B-form to A-form is reversible process, depending on the levels of sample hydration [9].

SSB and DSB yields were determined in dry DNA films irradiated by XUV laser pulses. A control sample for each series of the irradiation was placed into the vacuum chamber but was not irradiated. The remaining samples were irradiated at the same distance from the source by different number of pulses, screened by aluminium foils of different thicknesses. The energy density for each sample was calculated from the time progress in discharge current generated by the photodiode and corrected to the transmission of the particular aluminium foil.

Figures 3 and 4 show the yields of the different forms of DNA quantified in the gel, plotted on the ordinate as percentages of total amount of initial DNA as a function of energy density on the sample surface (i.e.,

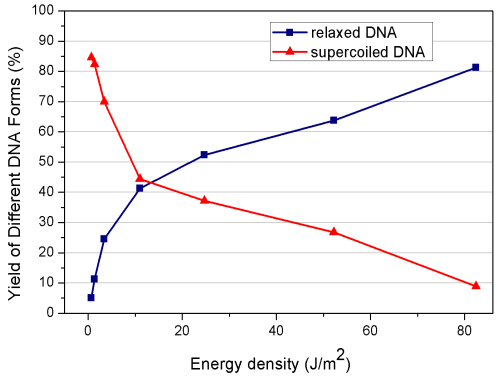


Fig. 3. Loss of supercoiled DNA and yields of SSBs as a function of energy density on the sample surface irradiated behind 0.15-µm aluminium shielding foil.

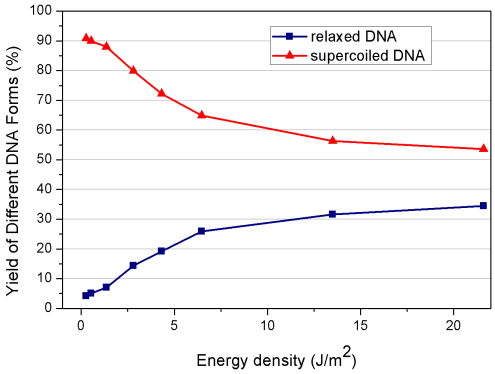


Fig. 4. Loss of supercoiled DNA and yields of SSBs as a function of energy density on the sample surface irradiated behind 0.4-µm aluminium shielding foil.

behind 0.15-µm and 0.4-µm Al foil), respectively.

The yield of the different forms of DNA irradiated without using of the Al filters is plotted in Fig. 5 as percentages of total numbers of initial DNA molecules versus energy density on the sample surface. We observed that the quantity of surviving undamaged supercoiled DNA decreases with increasing XUV energy density on the sample surface in a roughly exponential manner. The amount of supercoiled DNA increased with increasing exposures.

Comparing the results in the figures, in the experiment without attenuated radiation form XUV laser we have observed the production of linear form of the plasmid. We have not detected linear DNA in the samples irradiated behind Al foils at the same doses. The observed DNA SSBs damage presented in this paper can be assigned to 46.9 nm radiation exclusively due to the effective blocking of out-of-band radiation by aluminium foils. For unfiltered radiation the DSBs were present probably due to the influence of out-of-band radiation. At large XUV exposures, the decrease in supercoiled form of DNA is close to saturation, near 20%, which suggests that no more than 80% of the plasmids in the solid can be converted to either relaxed or linear DNA. Prise et al. [2] observed a similar exponential loss of supercoiled DNA with dose and a saturation from irradiation of DNA plasmids by low-energy photons. The yields of DNA SSBs and DSBs per base pair as a function of energy density on the sample surface for all three experiments (i.e., without Al foil and behind a particular Al filter) are presented in Fig. 6 (a,b), respectively.

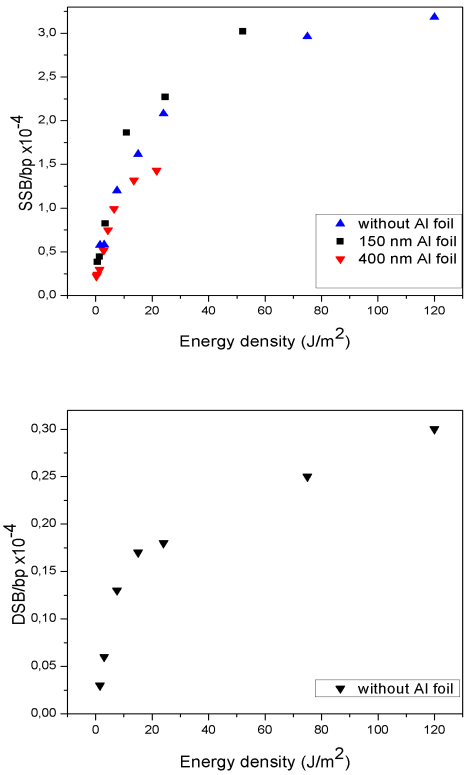


Fig. 6. Yields of (top) SSBs and (bottom) DSBs induced in pBR322 DNA plasmid per base pair induced by XUV laser.

The yields of SSBs and DSBs were determined from relative peak areas corresponding to the supercoiled (S), linear (L) and relaxed (R) forms of plasmid DNA separated on agarose gels. The yields of SSBs and DSBs were calculated as $G_{SSB}=\ln(1-L)/S$ and $G_{DSB}=L/(1-L)$, respectively, where $S+L+R=1$ [10]. The G(SSB) and G(DSB) for sample irradiated without Al filters are summarized in Table 1. The ratio of SSB and DSB yields was calculated to be 11.7 ± 3.2. For 1 keV radiation, the ratio of SSB and DSB yields

Energy density (J/m^2)	G(SSB) (breaks/bp x 10^{-4})	G(DSB) (breaks/bp x 10^{-4})
1.5	0.58	0.03
3	0.59	0.06
7.5	1.2	0.13
15	1.62	0.17
24	2.08	0.18
75	2.96	0.25
120	3.18	0.3
Control	0.12	0

Tab. 1. Yields of DNA Strand Breaks

was determined to be 8.7 ± 0.8 [5]. The found value is close to the value of 11 [11] determined similarly as in our study for 1.5 keV AlK_α X-rays and 10 obtained for γ-radiation [12].

CONCLUSIONS

Both SSB and DSB yields were determined in plasmid DNA irradiated by nanosecond pulses of 46.9-nm laser radiation. Obtained values of SSB yields clearly indicate that XUV CDL initiated chemical changes in DNA are more similar to that caused by an ionizing radiation rather than photo-modification due to ultraviolet illumination. However, DSBs appeared only when DNA was irradiated by both plasma emissions, i.e., coherent XUV laser beam and incoherent broadband UV-Vis emission. This finding is a subject of further investigation. Anyway, both yields determined here are very close to the values obtained with soft X-rays. An absolute re-calibration of vacuum photodiodes is in progress to confirm the agreement. In conclusion, XUV CDL has been proven as a source of ionizing electromagnetic radiation which is suitable for investigation of radiation damage to bio-molecular solids.

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