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Biological Action in and out of the Water Window

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This study is dealing with the difference of radiation chemical yields of single and double strand breaks induced in plasmid DNA by photons inside and outside of the soft X-ray water window, i.e., in the wavelength range from 2.28 nm to 4.88 nm. Photons were generated by various plasma sources providing nanosecond and sub-nanosecond pulses of extreme ultraviolet, soft X-ray and X-ray radiation. DNA strand breaks were determined by agarose gel electrophoresis. Higher radiation chemical yields of both single and double strand breaks were found using picosecond and nanosecond sources of extreme ultraviolet and X-ray radiation.

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1. Introduction

Short-wavelength sources have already reached the water window between the K-absorption edge of oxygen (543 eV) and carbon (283 eV). The fact that carbon atoms absorb radiation in this energy region more strongly than oxygen can be utilized not only for an imaging of living matter but also for a direct estimation of the role played by direct and indirect processes in an action of ionizing radiation on biomolecules in an aqueous environment. Direct action of the ionizing radiation on DNA is to a certain extent limited by the presence of the structural water attached to the DNA sugar phosphate backbone. Weaker absorption in water molecules in the water window spectral range results into an easier penetration of the radiation towards carbon rich bases and sugar molecules making the direct action more pronounced.

Experiments with cells or other complex models of biological structures found no evidence for a difference in relative biological effectiveness (RBE) in comparison between the conventional (usually quasi-continuous) and laser-driven and discharge (pulsed) sources of ionizing radiation; see for example [1–3]. Plasmid DNA however, allows for an easy detection of single (SSB) and double (DSB) strand breaks and remains its damaged structure intact for relatively long period if it is stored in a proper environment, being thus an appropriate candidate for dose rate studies.

This work is primarily focused on the study of the dose rate effect on radiation chemical yield (G value) of strand breaks in plasmid DNA induced by pulsed laser-plasma radiation. It is the successive experiment to the study performed earlier at Laserix [4] and PALS [5] facilities with picosecond (sub-nanosecond) pulses and using the gas puff target and table-top capillary-discharge laser (CDL) systems [6, 7] delivering nanosecond pulses pulses.

2. Materials and methods

Samples were prepared from the plasmid pBR322 (4361 bp, New England Biolabs, UK) stored in 1 × TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). Stock solution was further diluted with deionized water to make a working solution containing 0.04 × TE and plasmid DNA in concentration of 30 ng/μl. The amount of 5 μl of this solution was pipetted on a center of a glass coverslip (20 × 20 mm^2) and left to dry in a nitrogen filled dessicator for 60 min. Such obtained dry layers of DNA were subjected to irradiation in vacuum conditions. Samples were evaluated by means of agarose gel electrophoresis further described in [7].

Irradiation was carried out by pulsed plasma-based sources, both coherent and incoherent ones (Table I). Extreme ultraviolet (XUV) radiation (26.4 eV) was emitted by the compact CDL device described in [7]. 58.5 eV photons were delivered by Ne-like Zn laser driven by PALS [8]. Nanosecond water window radiation was generated using a table-top system described in [6]. PALS system was used to generate water window radiation on the sub-nanosecond time scale from Ar gas puff target. Xe gas puff was used to generate pulsed radiation with maximum at 1.2 keV also with sub-nanosecond time duration.

Average number of single and double strand breaks induced by unit energy fluence absorbed per plasmid, β_{SSB} and β_{DSB}, respectively, were found by fitting the integrated fractions of $S(\psi)$-supercoiled, $C(\psi)$-closed circular and $L(\psi)$-linear of plasmid forms as a function of energy fluence by the following set of equations:

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$$S(\psi) = S_0 f \exp\left[-(\beta_{\mathrm{SSB}} + \beta_{\mathrm{DSB}})\psi\right] + S_0(1-f), \quad (1)$$

$$C(\psi) = (C_0 + S_0 f)\exp\left(-0.5\beta_{\mathrm{SSB}}^2 \rho\psi^2 - \beta_{\mathrm{DSB}}\psi\right) - S_0 f \exp\left(-(\beta_{\mathrm{SSB}} + \beta_{\mathrm{DSB}})\psi\right), \quad (2)$$

$$L(\psi) = (C_0 + S_0 f)\left[1 - \exp\left(-0.5\beta_{\mathrm{SSB}}^2 \rho\psi^2 - \beta_{\mathrm{DSB}}\psi\right)\right] + L_0, \quad (3)$$

where S_0, C_0 and L_0 are the initial fractions of supercoiled, closed circular and linear forms of plasmid DNA. Parameter f is the maximum fraction of supercoiled form converted by the radiation and ρ is the probability to cleave the plasmid by two single strand breaks on opposing strands.

G value of SSB or DSB expressed in nmol J^{-1} was calculated according to

$$G_{\mathrm{SSB,DSB}} = \frac{\beta_{\mathrm{SSB,DSB}}\sigma f S_0 10^9}{m_{\mathrm{pBR322}} N_{\mathrm{A}}}, \quad (4)$$

where β_{SSB} or β_{DSB} are the fitted parameters based on the absorbed energy fluence for SSB and DSB respectively, σ is the surface density (5.8×10^{-5} kg m^{-2}), N_{A} is the Avogadro constant and m_{pBR322} is the plasmid mass which was taken to be, including structural water (2.5 water molecules per nucleotide), 5.11×10^{-21} kg.

3. Results and discussion

G values obtained with synchrotron radiation [9] and plasma sources in the present study are summarized in Fig. 1. It can be seen that the G values of SSBs are higher than those found with synchrotron radiation.

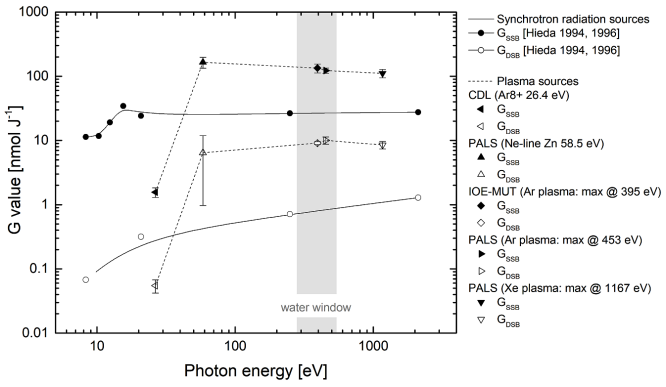

Fig. 1. G values found for monochromatic short-wavelength laser radiation (26.4 and 58.5 eV) and broad-band emission from laser plasmas generated in Ar and Xe gas puff targets compared to the G values obtained with the help of monochromatized synchrotron radiation. Water window spectral range is marked with the grey stripe. Lines are just guides to the eye.

As ionizing radiation can create strand breaks in DNA via direct action by sugar radical formation [10], residual water molecules can also be easily ionized to yield hydroxyl radicals which can abstract hydrogen from the base or sugar phosphate backbone. The latter reaction leads also to strand breakage via the indirect action [11]. When the radiation is delivered in a single pulse with a very short time duration (hundreds of ps to a few ns) the created radicals undergo recombination reactions due to the high density of ionizations more likely, lowering thus the probability of reaction with DNA subunits (bases, sugars).

Only a slight difference of the G values for both SSB and DSB can be seen in Fig. 1 for the radiation in the water window. When the peak energy of the plasma emission was shifted from 395 to 453 eV together with a decrease of the pulse duration τ, the value of G_{SSB} dropped from 134 to 122 nmol J^{-1}. Spectrally, this can be explained by higher absorption cross-section of nitrogen atoms above the nitrogen K-edge (> 410 eV) energy. Thus a higher fraction of radiation energy is absorbed in nitrogen-rich nucleobases. It seems that the direct action of soft X-rays on nucleobases does not enhance formation of SSBs and DSBs. The effect of different pulse duration can also contribute to the yield drop. We should take into account that the pulse lengths drop from 5 ns (IOE-MUT laser-driven Ar plasma with maximum at 395 eV) to 500 ps (PALS-driven Ar plasma with maximum at 453 eV). An analysis of the role of both factors by means of further experiments and computer simulations is in progress. Very low G value of both SSB (1.6) and DSB (0.06) at 26.4 eV, i.e. in a spectral range where the absorption of the DNA molecule is very high, is surprising. This might be possibly explained by selective absorption on nucleobases which leads to less strand breaks formation.

TABLE I

Plasma-based sources used in this study.

The source	E_{ph} [eV]	τ	ψ_{37}^{SSB}**	ψ_{37}^{DSB}**
CDL Ne-like Ar [7]	26.4	1.5	7.8	209.4
PALS Ne-like Zn [8]	58.5	0.5	0.1	2.5
PALS Ar plasma [5]	453*	0.5	4.2	51.5
PALS Xe plasma [5]	1167*	0.5	13.5	176.0
IOE-MUT Ar plasma [6]	395*	5	1.9	27.9

*A photon energy belonging to the intensity maximum in the spectrum.
**τ is pulse duration [ns]; $\psi_{37}^{\mathrm{SSB,DSB}}$ [J/m^2] are the entrance energy fluences required to induce single SSB or DSB per plasmid.

4. Conclusions

Very likely, both radiation wavelength and pulse duration play an important role in an action of electromagnetic ionizing radiation on DNA. In the water window, the direct action seems to be preferred but there might be a photon energy limit (K-edge of nitrogen) where nucleobase modifications begin to compete with the strand break formation. However, the correction to the different pulse duration of utilized pulses should be made to confirm this conclusion. These findings could exhibit an

importance for an imaging of carbon and nitrogen rich molecules (DNA, proteins) in the water window.

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