DNA damage by a single intense shot of soft X-rays emitted by a laser-produced plasma

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Abstract

A single-shot damage induction to plasmid DNA was demonstrated by applying X-rays emitted by a laser-produced plasma. Yields of single-strand breaks and double-strand breaks were determined as a function of energy fluence which was adjusted by varying the distance of the exposed sample from the X-ray source and by thickness of Al filters attenuating X-rays. As an intense source of X-ray radiation was employed a double-stream gas puff target irradiated by sub-kJ, near-infrared (NIR) focused laser pulses at the PALS facility (Prague Asterix Laser System) to produce high-energy pulses of soft X-rays from hot, dense Xe plasma. The double-puff arrangement ensures high gas density and conversion efficiency from NIR to X-rays approaching that typical for solid targets. In addition, its major advantage over solid targets is that it is debris free and has substantially suppressed charged particle emission.

INTRODUCTION

Soft X-ray radiation shows a limited application in a practical radiobiology due to its intense interaction even with low Z materials, i.e., air, water, tissue, which results in a very short attenuation length. However, the keV radiation can play an important role in the effort to understand the nature of radiobiological phenomena. Four main reasons for the study of radiobiological effects induced by soft X-ray radiation were identified in Goodhead's review [1].

The soft X-ray radiation makes the estimation of importance of low-energy electrons in biological action of all ionizing radiation possible. The low energy photons produce isolated tracks of electrons with small, well defined energies and very short tracks, comparable with size of critical genetic structures in cells such as DNA, nucleosomes and chromatin fibers [1, 2]. It is important that keV-photons can produce keV and sub-keV electrons only. Therefore, a pure experiment focused only on an action of low energetic electrons excludes the effect of γ rays and hard X-rays and, thus, the effect of high energetic electrons.

Although, the biological effects of low energy X-rays are currently studied using synchrotron radiation [3-10] in advantage due to the possibility to tune its wavelength, the laser-produced plasma as an X-ray source shows a high dose rate in contrary to the synchrotron sources. Therefore, only a single shot can be needed to induce a measurable biological effect induced by the radiation. We should note that the repair of radiation damages represents a source of difficulties in such studies.

The real-time concentration of radiation-induced species and excitations is usually low if the synchrotron radiation is applied. The genotoxic effects of soft X-ray singe-pulses from a laser-produced plasma were already studied by Shinohara et al. [11]. However, these studies were devoted to ~10 keV radiation rather than above-mentioned ~keV radiation. The laser-plasma soft X-ray source was used also by Hill et al. [12-13] to investigate temporal effects in irradiation of mammalian cells. This source cannot be employed as a single shot source because of small energy content in a pulse. Detailed description of such sources, driven by high-repetition lasers is given in a monograph [14].

The goal of our contribution is to introduce a new source of soft X-ray radiation for biological applications [15]. Preliminary experiments on DNA

damage caused by a single-shot of broad-band soft X-rays are reported. The irradiation of samples has been performed using Xe plasma produced by laser pulses. The single sub-nanosecond XUV/X-ray pulses having a wide continuous energy spectrum maximum intensity at 1.15 keV curried a total energy of ~ 100 J. The energy spectrum was modified using different gases or gas mixtures and X-ray filters.

EXPERIMENTAL SETUP

The XUV/X-ray source

The hot dense plasma, emitting sub-nanosecond XUV/x-ray flashes, was produced by focusing a near-infrared laser into a double stream gas puff target based on two coaxial nozzles [15]. The annular outer nozzle creates a hollow cylinder of helium, which hampers the sideways expansion of the central Xe gas stream injected in the system from an electromagnetic valve through a 2.0 mm diameter circular nozzle. The outer nozzle orifice is a ring with outer and inner diameters of 3.0 mm and 2.5 mm, respectively. Double-stream gas puffs have been demonstrated [16, 17] to improve NIR to XUV conversion radiation efficiency, compared to conventional single-stream gas puffs. For Xe central gas the conversion efficiency can reach 30% [16]. The X-ray streak camera (Kentech Instruments) checked the time shape of the emitted x-ray pulses. The undesirable ion emission, which could damage an irradiated sample, was eliminated by the outer He gas envelope surrounding the Xe plasma. Absence of ion current was monitored by ion collectors.

The x-ray source driver

The PALS facility is a high-power iodine photodissociation laser system which consists of a master oscillator, one pre-amplifier and five amplifiers [18]. At the fundamental frequency, the full amplification chain provides 1 kJ, 300-ps pulses of 1.3152 mm radiation. The NIR beam has a diameter of 29 cm, when leaving the laser system. Then the beam is guided in a spherical interaction chamber and focused 2 mm above the gas puff orifice.

X-ray source characterization

The energy of X-ray pulses was measured using two silicon photodiodes BPYP 03 type (for more details see ref. 19) located at a distance of 2.5 m from the plasma. The diodes were covered with filters enabling measurements of X-ray radiation in two different wavelength ranges: 5-μm Al for λ = 1.1±0.2 nm and 200-μm Be for λ = 0.26±0.02 nm. If needed, the radiation was attenuated using a fine metallic mesh placed between the plasma and detector. The conversion efficiency of the NIR laser pulse energy into 1.1-nm X-ray radiation was estimated to be typically of 15-20% and in the wavelength range close to 0.26 nm was over 100 times less.

Soft x-ray spectra were measured using the transmission grating spectrograph (4000 lines/mm) and the back illuminated CCD camera (Reflex s.r.o.; Czech Republic). Resolution of the spectrograph was 0.15 nm what was too low to resolve thousands of Xe plasma lines close to each other.

DNA plasmids preparation and strand break analysis

The plasmid pCDNA3 (5446 base pairs) was transformed into JM109 host strain of E. coli (ChemosCz) by thermic chock (1-2 minutes at 42 ° C followed by 2 minutes on ice).

Several chosen colonies grown on Petri dish filled with LB/agar culture medium with 100 mg/ml ampicillin were isolated and left to grow in 2ml LB medium with ampicillin.

The plasmids were then isolated using GenElute Plasmid Miniprep kit (Sigma).

The colonies containing plasmids pCDNA3 were subsequently grown in 100 ml LB medium with Ampiciline for 24 hours and then plasmids were purified using GenElute Plasmid Maxipred kit (Sigma) and stored at –20 ºC in 10mM Tris-HCl, pH=8.0, 1mM EDTA buffer.

Samples of 10 ml containing 1 mg of pCDNA3 in 10 mM KCl and 100 mM phosphate buffer (pH=7.4) were deposited on glass plates and left to dry on air. The dried samples were then placed into the irradiation chamber at different distances from the source and shielded by an Al foils of a chosen thickness. The irradiated samples were re-dissolved in 20 ml of distilled water.

The thickness of samples was checked using measured with a surface profiler (Alpha-Step 500, Tencor, USA). The relatively homogeneous layer of dry DNA with thickness about 300 nm is surrounded on its periphery by circular mound thick about 650 nm. The sample thickness, density and elemental composition are important for calculating the fraction of X-ray pulse energy deposited in the DNA layer.

The irradiated and referential (non-irradiated) samples containing about 150 ng DNA were analyzed on 0.8% agarose gels, run in 0.5 TAE buffer at 100 V for 4 hours in presence of Sybr Green stain (Sigma, concentration 1:10 000). The final pictures were taken on the UV transilluminator table UVT-20ME by a digital camera Olympus C-720.

RESULTS

The yields of single-strand breaks (SSB) and double-strand breaks (DSB) were determined from relative peak areas corresponding to supercoiled (S), linear (L) and relaxed (R) forms of DNA plasmid separated on agarose gels. The yields of SSB and DSB were calculated as $G_{SSB} = \ln [(1-L)/S]$ and $G_{DSB} = L/(1-L)$ respectively, where S+L+R=1 [20]. A referential sample for each series of irradiation was placed into the vacuum chamber and protected from the irradiation; remaining samples were irradiated at different distances from the source and were screened by Al foils of different thickness. The applied fluence ψ was determined for each sample by the calibrated Si photodiodes which response was corrected to the spectral transmission of the applied Al foils. The applied fluence ranged from 10 J m^{-2} to ~ 100 J m^{-2}.

The fluence dependences of SSB and DSB yields per plasmid are presented in Figure 1. The corresponding ratio of SSB and DSB yields is 8.7□ 0.8. The number of the SSB and DSB breaks of DNA per plasmid increases linearly with the increasing fluence up to 175 J m^{-2}: $(29 \pm 1)\times10^{-3}$ SSB/plasmid/Jm^{-2} and $(3.4 \pm 0.1)\times10^{-3}$ DSB/plasmid/Jm^{-2}, respectively.

The substantial decomposition of plasmids occurring at higher fluences does not leave any undamaged DNA molecules in the sample. Therefore, the SSB yields cannot be determined at a high fluence level. The DSB yields can be determined, however, their values became underestimated.

DISCUSSION

The yields of SSB and DSB formation determined for 1 keV radiation emitted by our X-ray source correspond very well to the value of about 30 nmol J^{-1} for 20 - 2000 eV X-rays [3] and 250 eV and 380 eV [21]. For 1.5 keV $Al_{K\alpha}$. X-rays, the value of 62 nmol J^{-1} has been determined [22]. Numerous papers, reviewed in Cai et al. [22], demonstrated further a SSB yield increase with the continuing increase of photon energy.

As for the SSB/DSB ratio, the value of 8.7±0.8 determined in our experiment is being close to the value of 11 for 1.5 keV $Al_{K\alpha}$ x-rays [22]. The comparable value of about 10 was obtained also for γ-radiation [23]. Slightly higher values were determined by Yokoya *et al.* [24] for a calf thymus DNA using 388 eV, 435 eV and 573 eV synchrotron X-rays - about 18 SSB/DSB, by Folkard *et al.* [6] for a freeze-dried pMSG-CAT plasmid using 100 eV synchrotron radiation - 19 SSB/DSB and by Hieda [3] for an air-dried pBR322 plasmid using 2 keV photon irradiation - 25 SSB/DSB. The differences of determined ratios can be influenced by the presence of remaining water and salts in the dried samples.

The plasma based on laser-irradiating the double stream gas puff target clearly demonstrated its capability to be an X-ray source suitable for the creation of a measurable number of both the single- and double-strand breaks in a thick plasmid sample by a single pulse. This encourages prospective utilization of the source in various pulse-probe schemes. Such a scheme will be used for time-resolved studies of reactive intermediates formed in the sample by sub-nanosecond X-ray pulse probed by the absorption and/or scattering of UV, vis or NIR laser impulse coming with a chosen delay after the ionizing radiation pulse. The main advantage of our irradiation layout lies in the fact that the time resolved results can be correlated with the yields of irreversible changes in the sample.

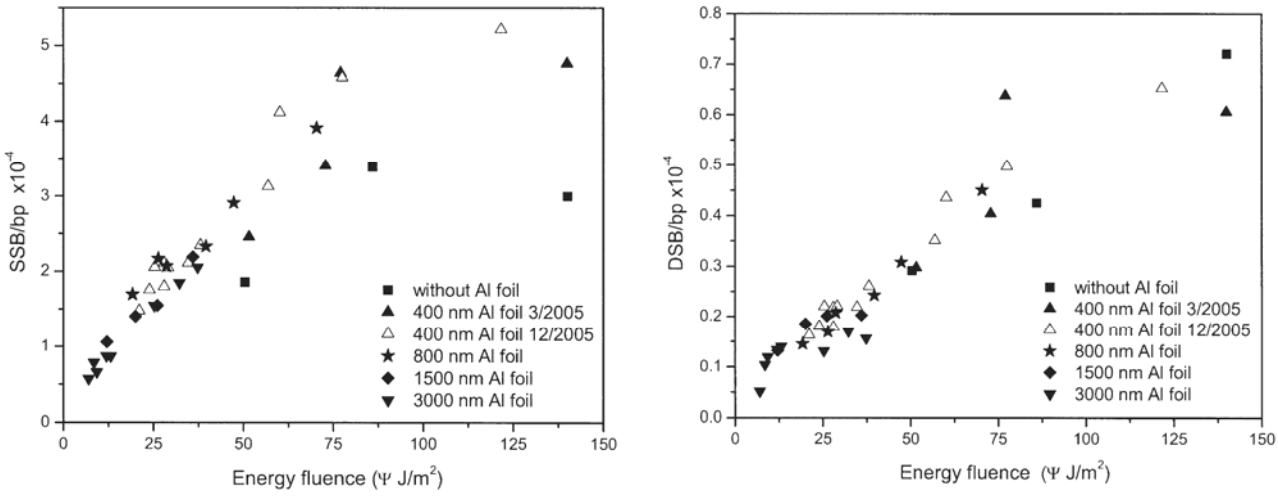

Fig. 1. Yields of SSB (left) and DSB (right) induced in pCDNA3 plasmid by a single shot from the double-gas puff source.

CONCLUSIONS

The determined values of yields of SSB and DSB formation as well as the value of SSB/DSB ratio were found in a very good agreement with experimental results of others research groups using the soft X-ray tubes and synchrotron radiation, i.e., applying much lower dose rates. Therefore, the plasma source, driven by a high-power laser system, demonstrated its ability to induce the measurable radiobiological change by an action of even a single shot. This allows using the source in prospective pulse-probe experiments.

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