Damage of supercoiled DNA by an ultrafast laser-driven electron x-ray source

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Abstract: Using magnetic fields to differentiate the effects of electrons and x-rays, it was discovered that single strand breaks in supercoiled DNA were mainly caused by the energetic ultrafast electrons rather than the ultrafast x-ray photons emitted from the same table-top ultrafast x-ray source. At constant pulse energy of the driving laser pulses, shorter laser pulses produced more strand breaks than longer ones. This was attributed to the increased flux of electrons produced with the shorter laser pulses. Other factors contributing to the DNA damage were investigated and discussed.

OCIS codes: (170.7160) Ultrafast technology; (320.7120) Ultrafast Phenomena; (350.5610) Radiation.

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1. Introduction

Ionizing radiation such as x-rays and electrons damages DNA mainly by causing single strand and double strand breaks (SSBs and DSBs).[1-3] These breaks can be produced via direct pathways such as direct ionization of bonding electrons in DNA base pairs or phosphate groups, and indirect pathways such as hydrogen-atom abstraction of deoxyribose moieties by hydroxyl radicals created from electrons interacting with water molecules. Electrons are in general more effective than x-rays of the same energy in terms of breaking DNA strands, mainly because the former can interact more effectively with DNA and water. As a result, the maximum linear energy transfer (LET) from radiation to DNA is higher for electrons than x-rays of the same energy, although more energetic electrons may have the same LET as the lower energy x-ray counterpart. For example, 400 keV electrons have approximately the same LET as 10 keV x-rays.

In general, the investigation of the effect of ionizing radiation on DNA strand breaks is performed with continuous sources of electrons or x-rays. However, because the time it takes to produce hydroxyl radicals and solvated electrons is very short, of the order of 10^{-13} to 10^{-14} seconds, it is desirable to create x-ray or electron pulses of such short duration to investigate these primary species. In the past, the shortest x-ray pulses were of the order of 100 ps and the shortest electron pulses were a few picoseconds long, preventing direct experimental investigations of the ultrafast phenomena and leaving most studies to rely on theoretical predictions. Only recently have ultrafast x-ray pulses and electron pulses been created by ultrafast laser pulses interacting with gaseous or solid targets.[4-7] The driving laser pulses can be as short as tens of femtoseconds, and in principle ultrafast x-ray and electron pulses of the same length may be generated.[8] In one kind of such sources, for instance, electrons are accelerated to many kiloelectronvolts (keV) to even a few megaelectronvolts (MeV) in a focused intense laser field.[9] When these electrons interact with a solid target such as copper wires or tungsten rods, hard x-rays are produced. Because the acceleration is achieved via a ponderomotive potential created by the intense and short laser pulses over a very short distance (a few tens of microns), the duration of the electron and x-ray pulses is very short at the focus as well. The conversion efficiency from electrons to x-rays, however, is generally very low, typically of the order of 10^{-3} to 10^{-5}.[9] Therefore, these ultrafast x-ray sources naturally also emit intense electron pulses. Because of the speed distribution in and repulsion between electrons, the pulse duration of these electrons increases as they travel away from the focus. On the other hand, the pulse duration of x-rays remains the same as generated. In the past, radiolysis of water has been performed with one of those sources, although no ultrafast measurements were performed due to the relatively slow instrumental response.[10] To date no investigations have been done on the effect of DNA strand breaks with these pulses.

If DNA is directly exposed to such a source, and if both electrons and x-rays are allowed to reach the DNA sample, then it is possible that both types of radiation can cause strand breaks in DNA. Because the flux of electrons in these sources is far higher than that of x-rays, it is expected that most of the DNA damage may be caused by the electrons. In this report, we wish to present the results of the first such investigation by comparing the damages caused by the electron pulses and x-rays pulses emitted from the same ultrafast x-ray source. In addition, we will also compare the yields of x-rays and electrons from the ultrafast source to conventional continuous wave sources.

2. Experimental Section

The experimental arrangement, which includes an ultrafast laser, an x-ray target, a sample, and an x-ray CCD (LCX-LN CCD-576, Princeton Instruments, Roper Scientific) is shown in Fig. 1. The ultrafast laser and x-ray target apparatus were described elsewhere.[11-13] Supercoiled DNA (scDNA) samples were contained in plastic vials (PCR tubes). Each sample of 20 μl scDNA aqueous solution with 200 μM Tris and 100 μM NaCl (Invitrogen, San Diego, CA) was loaded into the cap of the vial. Because 10 keV x-rays or 400 keV electrons can only penetrate ~1-mm of water, the sample thickness was maintained to be ~1 mm. The

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samples were wrapped in 2 layers of Al foil (16.25 μm per layer), and the sample-source distance was 15 cm. When needed, a pair of 2000-gauss magnets (Model 0005, ForceField, CO) separated by 1 cm were positioned between the x-ray source and the sample so that x-rays could pass in between them. Pb blocks were also used when conducting the beam size tests. In this case, a 5-mm hole was drilled in a 5-cm thick Pb brick. The brick was then aligned so that x-rays passed through the hole to reach the sample.

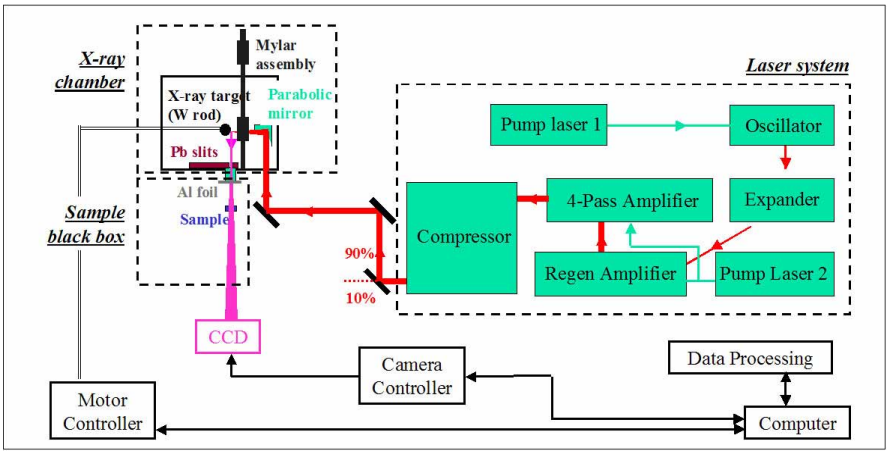

Fig. 1. Experimental layout. X-rays and electrons are produced from the x-ray target and collimated with the lead slits.

To generate the shortest optical pulses, the grating separation was optimized using an autocorrelator.[11] To lengthen the optical pulses, the grating separation was dialed a few millimeters away the optimal position. The CCD was used to measure the energy spectra of the x-rays at different grating separations. It has a spectral response range of 2 to 30 keV, although x-rays with higher energies of up to 50 keV were measured. Damage to the scDNA was measured with Agarose gel electrophoresis.[14] The cast gels (Invitrogen, CA) were operated at 45 V for 45 min, and then inspected with a transilluminator (ChemiDoc XRS, Biorad). In cases where a conventional x-ray radiation source was needed, a fixed-anode tungsten x-ray radiation unit (HP FAXITRON 43855A) was used. The operating voltage was 100 kV and the current was 3 mA.

The pulse duration of the optical pulses was measured with the autocorrelator when the pulse duration was estimated to be shorter than 100 fs.[11, 15] Pulse duration of longer optical pulses was estimated based on the dispersion of the compressor. A ion chamber handheld meter (RSO-50E, Bichron) was used to monitor the x-rays (up to a few hundred keV). This detector has a thin Al film cover, which blocks UV light and low energy electrons.

3. Results

The optical pulses were measured, and results were shown elsewhere.[11] The FWHM of the shortest pulse was 35 fs when the grating separation in the compressor was optimized. The duration became 2-4 ps when the grating separation was set to 4 mm away from the optimal distance. Under this condition, the pulses were chirped. X-ray spectra were obtained with the CCD for these two pulse durations. Fig. 2 shows the processed data. The original data was obtained in the single photon mode. The slopes of the two plots for these two different pulse durations represent the equivalent electron temperatures, which show that the electron temperature was higher for the 30 fs pulses (gentler slope). Using the formula given by Wilks et al., we estimated the electron temperature to be 100 keV for those created by the shorter laser pulses.[16] The estimated electron temperature was 25 keV for the longer pulses. The two measured temperatures were ~50 keV and 15 keV. Other formulae were also available. For

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example, using the formula obtained by Reich et al the electron temperatures were estimated to be 400 keV and 40 keV.[17] All these results suggested that shorter optical pulses produced more high energy electrons and thus more high energy x-rays, which were measured here.

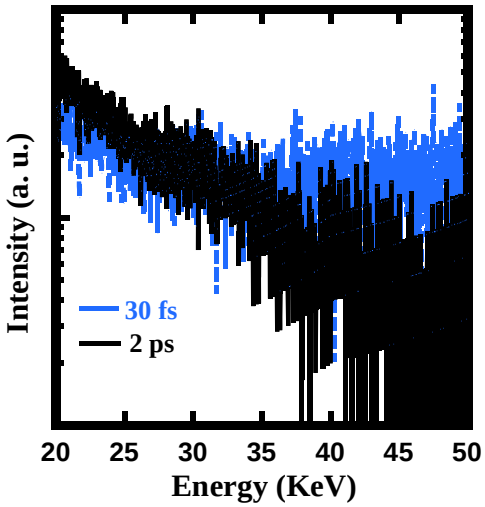

Fig. 2. X-ray spectra at shorter and longer optical pulses. The slope indicates the electron temperature. The dark line represents the x-ray photon histogram of the longer pulse, and the gray line of the shorter pulse.

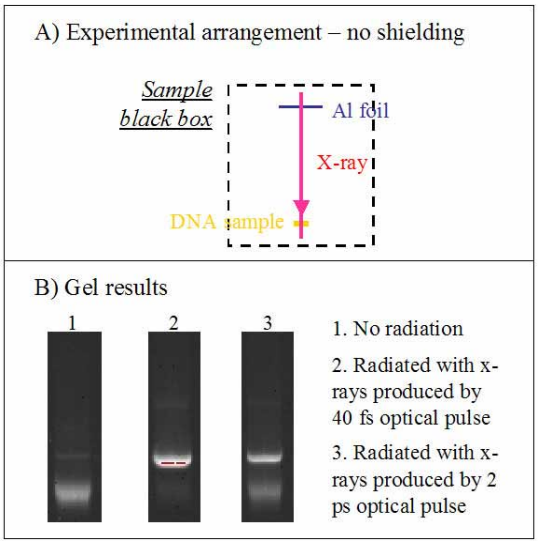

Fig. 3. DNA damage by LEXS. Fig. 3A shows the arrangement of the sample with respect to the source. Fig. 3B shows the gel results. The top band in each lane represents the damage scDNA, and the lower band is the scDNA.

When the DNA samples were exposed to the x-ray source that produced short or long pulses, the extent of strand breaks and the resulting amounts of relaxed or damaged scDNA were different. Fig. 3A shows the arrangement of the sample with respect to the source. Fig. 3B shows the DNA damage by pulses of two durations. The results clearly showed that shorter optical pulse generated more x-rays and electrons and thus gave rise to more scDNA

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damage (89% damaged in Lane 2 of Fig. 3B versus 53% in Lane 3). Results from the conventional continuous wave x-ray radiation unit were used to calibrate the equivalent x-ray dosage that causes this damage, assuming no nonlinear effects existed when short pulses were used. Based on the calibration, the equivalent x-ray dosage to cause the same damage shown in Lane 2 of Fig. 3B is 2 Gy. It is worth pointing out that this estimation only revealed how much x-rays were needed to cause this damage. Because 400 keV have the same LET as 10 keV x-rays, it is possible that the electrons actually caused all the damage, as shown later. Because of the Mylar foil, two layers of Al foil (to block the UV light and low energy x-rays and electrons) and 5 cm of air, only electrons with kinetic energy over 60 keV or x-rays over 5 keV can reach the DNA sample. Taking these parameters into consideration, the DNA samples were damaged by either 60 – 1000 keV electrons, or 5–14 keV x-rays, or both.

In order to differentiate the role of electrons versus x-rays, magnetic fields were introduced between the samples and the x-ray source. The arrangement is shown in Fig. 4A. The gel results are shown in Fig. 4B. It is evident that DNA damage under shorter optical pulse excitation was greatly reduced with the installation of the magnets, as all three lanes in Fig. 4B now show little damage to the DNA. The same occurred to the longer optical pulse case. The strength of the two magnets was 2000 gauss. Taking the geometry into consideration, the estimated cyclotron radius was smaller than 1 cm for 500 keV electrons. This suggested that most electrons would not be able to reach the DNA sample if their energy was lower than 500 keV. This result clearly demonstrated that majority of the damage in both cases were caused by the electrons.

We also tested the beam size effect, as it is known that broad beam may generate secondary x-rays or electrons that could amplify the damage. In this case, the Pb block was used to create a collimated x-ray beam with the same size as that of the sample. Compared to measurements without the lead block, the damage to scDNA was reduced by less than 10%, much less than the measured decreases when magnets were used. Therefore, there were little secondary x-rays or electrons generated around the target chamber or near the samples.

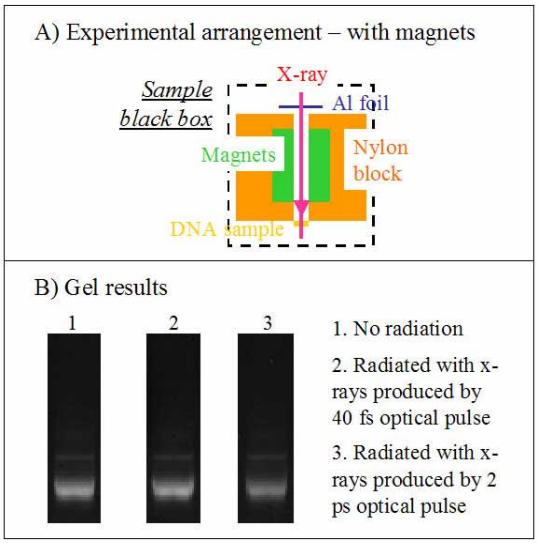

Fig. 4. DNA damage by LEXS with magnetic field shielding. Fig. 4A shows the arrangement, and 4B shows the gel results. Little damage to scDNA was observed in all three cases.

We studied the DNA damage with a thick Al plate filter (1 mm) as well. With the Al plate, almost no DNA damage was observed using either long or short laser pulses. Because this plate can totally attenuate electrons up to several MeV, but can only block x-rays up to 50

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keV, the results suggested that the observed DNA damage in Fig. 3 was caused by the energetic electrons and not by high energy x-rays (>50 keV).

5. Discussion

The x-ray yield was monitored with the Bichron handheld survey meter, which can detect electrons (>100 keV) and x-rays (>12 keV). When placed behind thick Pb blocks, the meter had a higher reading for shorter laser driving pulses. This suggested that such responses were mainly caused by high energy x-rays, possibly MeV in energy because all the electrons should be stopped by these lead blocks. However, although these x-rays were present, they were too few to cause damage to the DNA in the small sample holders. Because of the attenuation through the distance and the lead blocks, the photon density was extremely low at the survey meter. In this case, the meter simply detected single or a few high energy x-ray photons. For the ultrafast x-ray pulses at 50 Hz repetition rate and the meter with a response time of a second, the pulsed x-ray source acted as a low yield γ–ray source to the detector.

It is also possible that pulse duration of the electrons emitted from the source at those two different conditions contributed to the difference between the observed damages in Lane 2 and 3 in Fig. 3B. However, this is unlikely for two reasons. First, as shown in Fig. 2 there were more high energy electrons (>60 keV) in the shorter pulse case that could reach the DNA sample. Because the damage was mainly caused by these electrons, more electrons should generate more strand breaks. Second, the pulse duration of electrons, even excited by the shortest optical pulses, may still be of the order of tens of picoseconds due to the dispersion of electrons of different speeds traveling in space. It is unlikely that such a long pulse could cause any nonlinear damage to DNA.

In comparison to the conventional x-ray source, the absolute average yield of x-rays from this ultrafast x-ray source was about three orders of magnitude lower. However, taking into account of the electrons emitted from the ultrafast source, the equivalent dosage would be the same as the conventional x-ray source. The relative efficiency of x-ray generation, however, is the same for the two sources, which was of the order of 10^{-4} (defined as x-ray power output divided by laser or electron power input).

It is possible that electron pulses of different durations that have the same overall flux can be produced to test the effect of pulse duration effect when the electron pulses are shorter than 1 picosecond. When the pulses are longer, such as the cases discussed here, nonlinear effects may not occur due to slow diffusion processes involving solvated electrons and hydroxyl radicals generated from these electrons. When the pulse duration of the electrons is comparable to that taken to generate solvated electrons, nonlinear effects may be observed. The samples must be placed very close to the source for this to happen.

The results presented here clearly demonstrated that both electrons and x-rays are emitted from these ultrafast x-ray sources, and the flux of electrons is much higher than that of x-rays near the source. It is possible to study the nonlinear x-ray dose-effect relationship with these sources because these x-ray pulses can be shorter than 1 picosecond. However, as it is shown here, that the x-ray flux is low, and electron effect must be eliminated when placing the samples close to the source.

6. Summary

Radiation induced strand breaks in supercoiled DNA were used to demonstrate that ultrafast laser driven electron x-ray sources emit both ultrafast x-ray and electron pulses. When unattenuated, the flux of the energetic electrons can be several orders of magnitude higher than that of the x-rays.

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