Research Group for Radiation and Biomolecular Science

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The research objective of our group is to elucidate the radiation effect of living systems from molecular to cellular level. Taking advantage of beams obtained from synchrotron radiation, high energy-resolution as well as parallel beam, we examined following two subjects: (1) DNA damage induction by oxygen *K*-shell excitation, and (2) effect on cell cycle induced by single cell irradiation with X-ray microbeam.

DNA damage induced by oxygen *K*-shell excitation

Previously we revealed that yields of base lesions as well as single strand breaks (SSBs) arising in DNA were significantly enhanced by oxygen *K*-shell ionization [1]. In this study we examined the effect of oxygen *1s* electron excitation to π* anti-bonding orbital on DNA damage induction. The damage process by the excitation resulting in a singly-ionized DNA molecule is thought to be different from that for *K*-ionization resulting in a doubly-ionized molecule.

Plasmid DNA films were exposed to soft X-rays (BL23SU, SPring-8) in a vacuum at the π* peak (531.6 eV) and above oxygen K-ionization energy (560 eV), and then recovered with a buffer. Base lesions or abasic (AP) sites produced were converted to SSBs by a treatment with specific glycosylases. The enzymatically induced SSBs, as well as that for SSBs directly induced by irradiation, were analyzed by agarose gel electrophoresis. We used Fpg and Nth, recognizing purine and pyrimidine base lesions respectively, for the enzymatic treatment. These base lesions are hereafter denoted as Fpg- or Nth-sensitive sites. Nfo was used to convert AP sites to SSBs.

Although the X-ray absorbance at π* peak is similar to that at 560 eV, yields of each damage induced by the π* excitation were significantly larger than those obtained by irradiation at 560 eV (Fig. 1). Particularly the yields of SSBs and Fpg sensitive sites, which is mainly oxidative purine lesions such as 8-oxoguanine, were about 1.6-fold of those for 560 eV irradiation. The yields of Nth sensitive sites (mainly pyrimidine lesions such as dihydrothymine) and AP sites showed relatively smaller values than those for SSBs and Fpg sensitive sites (half or less). These results suggest that the excitation state with an electron in anti-bonding π* orbital significantly contributes to the production of SSBs and purine damage [2].

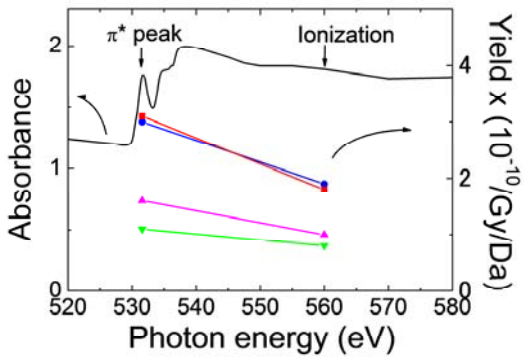

Fig. 1. Photo-absorption spectrum of DNA films (solid line) around oxygen K-shell edge and yields of SSB (●), Fpg (■) or Nth sensitive site (▲), and AP sites (▼) induced in plasmid DNA by soft X-ray irradiation, respectively.

Cell-cycle-selective irradiation to single cell with X-ray microbeam

In order to understand the mechanism giving a radiosensitivity depending on cell cycle, study of the biological effect on cells irradiated at a specific cell cycle stage has been desired. Recently, fluorescent ubiquitination-based cell cycle indicator (FUCCI) has been developed as one of probes to visualize the cell cycle as live cell images [3]. We investigated the live cell images of FUCCI-HeLa cells (red indicates G1; green, S/G2) exposed to synchrotron X-rays obtained by a time-lapse method.

FUCCI-HeLa cells on a dish with a polyester film bottom were irradiated by synchrotron X-ray microbeam (BL27B, Photon Factory, KEK). The beam size was adjusted to 20 μm × 20 μm square. After selective irradiation to cells at G1 or S/G2 stage with a dose of approximately 5 Gy, the dish was removed from the online microscope system and set to the offline one. To track the progression for cell cycle of irradiated cells, we constructed an off-line time-lapse system, which equipped with a small incubator on the stage. The cell images were snapped every 2 hr by a CCD camera for 24 hr (Fig. 2). In order to confirm the formation of double strand breaks (DSBs), immunostaining of phosphorylated histone proteins (histone γ-H2AX) was also performed.

Using this system, we obtained following results. The cell cycle was strongly arrested by irradiation at S/G2. In contrast, cells irradiated at G1 progress into S/G2 with a similar time course as non-irradiated control cells. It suggests that the G1-S (or S) checkpoint pathway does not work in HeLa cells, even though X-ray microbeam irradiation significantly induces DSBs. Presumably after DNA replication, the cell cycle is arrested by the S or S-G2 checkpoint [4]. These results show single FUCCI cell exposure and live cell imaging are powerful methods for studying radiation effects on the cell cycle.

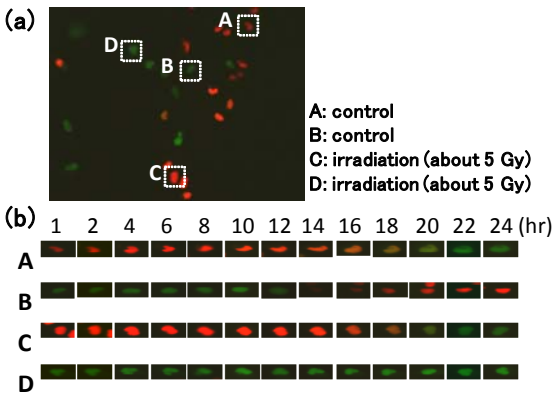

Fig. 2. (a) Image of FUCCI-HeLa cells before X-ray microbeam irradiation and (b) results of temporal observation over 24 hr for the cells labeled A, B, C, and D. A and B cells were not irradiated (controls), while C and D cells were irradiated.

References

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