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# Chromatid-type aberrations induced by monoenergetic soft X rays with resonance energy of phosphorus K-shell absorption edge

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## 1 Introduction

We reported the effect for chromatin-break induction in Chinese hamster lung fibroblast-like cells (V79) irradiated with monochromatic soft X rays near K-shell absorption edge of phosphorus, which is one of major elements formed DNA backbone. Monochromatic soft X rays were produced using an InSb double-crystal monochrometer at beam-line 27A in Photon Factory, KEK. Chromatin-break induction was measured by counting the number of chromatin fragments at $G_1$ phase detected by the premature chromosome condensation (PCC) technique. The results indicated that about 60% of initially measured chromatin breaks induced by the X rays at 2146eV and 25% of breaks induced by the X rays at 2160eV rejoined after 16 hours of post-irradiation incubation, while only 3% of repairable breaks were observed at 2153eV [1]. This repair value of 60% was the same level with high linear-energy-transfer (LET) carbon-ion beams with 100 to 150 keV/µm. We concluded that there produces much non-rejoining chromatin breaks by the inner shell absorption of phosphorus. The data indicated the quantitative differences in induced chromatin breaks near K-shell absorption edge of phosphorus, however, it is still unclear what kinds of qualitative differences in cellular damage between K-shell absorption edge and out of edge are. In this study we tried to identify qualitative differences in induced chromatin aberrations near K-shell absorption edge of phosphorus using a chemical induced $G_2$-PCC technique.

## 2 Experiment

Normal human skin fibroblasts distributed by the RIKEN BioResource Center Cell Bank (Cell name, NB1RGB; Cell No., RCB0222) were used in this study. Cells were cultured in Eagle's minimum essential medium (MEM) containing kanamycin (60 mg/l), supplemented with 10% fetal bovine serum in a 5% $CO_2$ incubator at 37 $^{o}$C. The frozen stocked cells were thawed in a water bath at 37°C for irradiation, then inoculated and subcultured in the 75-cm$^2$ plastic flask until they reached a confluent state. The cells were trypsinized and inoculated onto irradiation dishes. The following day, medium in the dishes was removed by aspiration and the dishes were covered with 5-µm thick polyester PET film for monochromatic X-ray irradiation.

The cells were irradiated 500R with monochromatic X-rays of 2146, 2153 and 2160eV, which were monochromatized with an InSb double-crystal monochrometer. The calibration of energy for resonance of K-absorption peak (2153eV) of phosphorus was carried out using the absorption spectrum of a DNA film [2]. The dosimetry of the exposure (R) was carried out using a free air ionization chamber [3]. It was converted the exposure (R) into the absorbed dose (Gy) using the mass energy-absorption coefficient of air and the mass attenuation coefficient of cell [4]. We calculated the conversion factors (Gy/R) to be 0.0085949 for 2146eV, 0.0088479 for 2153eV and 0.0086908 for 2160eV, respectively.

Immediately after irradiations, the irradiated cells were treated with Calyculin A (Wako Chemicals, Tokyo, Japan) at a final concentration of 50nM for 30 min in a $CO_2$ incubator at 37$^{o}$C. The PCC samples at a $G_2$ phase were prepared according to a conventional cytogenetic procedure [5]. Briefly, cells were treated with a 75mM KCl solution for 20min at 37$^{o}$C, and fixed in 3:1 methanol : acetic acid. The cell suspension was dropped onto ethanol-cleaned slides, air dried, and stained with a 5% Giemsa solution. The PCC samples of 50 $G_2$ phases were scored under a light microscope. The yield of induced chromatid-type aberrations was determined as previously reported by Savage [6]. We scored chromatid breaks, gaps, isochromatid deletions, and exchanges as chromatid-type aberrations.

## 3 Results and Discussion

The ratios of DNA absorbance near K-shell absorption edge of phosphorus based on the value at 2146eV were to be 3.36 at 2153eV and 1.43 at 2160eV [7].

The results were summarized in Table 1. The values of any categories of observed chromatid-type aberrations were higher at 2153 and 2160eV, which were in the side of higher energies at K-shell absorption edge of phosphorus, than at 2146eV. Especially the ratios of chromatid-type breaks and exchanges were much higher than those of the DNA

absorbance between lower energy and higher energies of K-shell absorption edge. The data suggests that chrotmatid-type aberrations are produced other process(es) of the DNA absorbance, such as the effect of Auger electrons. Also, isochromatid breaks did not produce at 2146eV. They must be produced by 2-hit events close to both single strand DNAs at the same time. The data, which shows the values of isochromatid breaks at 2153 and 2160eV, indicates that dense energy depositions occur near phosphorus in DNA strand at the higher energies of K-shell absorption edge of phosphorus, looking like the damage caused by high-LET radiations. The results in this study suggest that energy depsition at K-shell absorption edge of phosphorus enable to induce serious damage in DNA/chromatin level, which are similar to high-LET radiations.

Table.1 Chromatid-type aberrations in normal human fibroblasts immediately after irradiations

| Energy | Chromatid-type aberrations per cell | | | |
|---|---|---|---|---|
| (eV) | Gap | Break | Exchange | Isochromatid break |
| Control | 0.10 | 0.10 | 0 | 0 |
| 2146 | 0.30 | 1.2 | 0.05 | 0 |
| 2153 | 0.45 | 5.9 | 0.64 | 1.7 |
| 2160 | 0.36 | 3.2 | 0.21 | 0.78 |

The exposure of each energy was 500R. The absorbed doses were calculated using the conversion factors (Gy/R) to be 4.297Gy for 2146eV, 4.424Gy for 2153eV and 4.345Gy for 2160eV.

References

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