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Base damage induced in hydrated plasmid DNA by phosphorus K-shell photo-absorption as detected by base excision repair enzymes

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Introduction

Monochromatic ultrasoft X-rays at around 2 keV are an attractive radiation source for selecting an exclusive K-shell photoabsorption on phosphorus in DNA, followed by the ejection of low-energy secondary electrons. Several studies have highlighted the yield of double strand break (dsb) relative to that of single strand break (ssb) in dry DNA using photons of ~2keV [1, 2].

The present study investigates the induction of oxidative DNA base lesions in **hydrated** plasmid DNA by phosphorus K-photoabsorption. The base lesions were visualized using base excision repair enzymes. Non-dsb containing clustered damage sites were also visualized as additional dsb by treatment with enzymatic probes.

Materials amd Methods

Plasmid DNA (pUC18) was obtained from and *E.Coli* HB101 host and extracted using alkali-lysis, as described in [3]. The freeze-dried DNA films were hydrated using a sodium hydroxide solution of 3.84 mol dm^{-3} to give a relative humidity of 97 % at 5.7 ºC as described previously [3].

The DNA samples at 97% humidity were put into a copper irradiation vessel (Fig. 1) with a NaOH solution and sealed to maintain the humidity of the sample during the irradiation. The temperature during irradiation was controlled at 5.7 ºC using a chiller thermocirculator. The irradiation experiments were performed using BL27A. Three photon energies, 2147 eV (below phosphorus K-edge), 2153 eV (on resonance) and 2160 eV (above the K-edge) were used for irradiation. To achieve uniform doses, the vessel was scanned on the beam axis. The maximum dose was estimated to be 10 kGy.

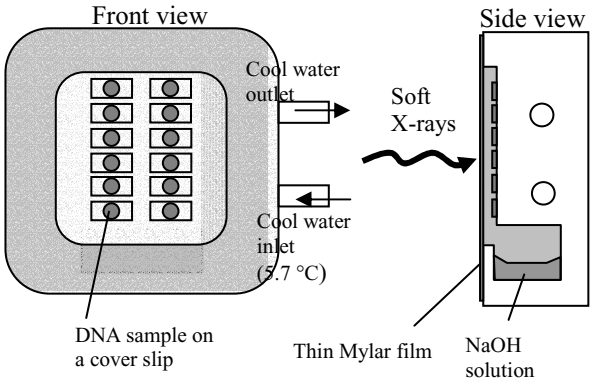

Fig. 1 Schematic layout of the vessel for ultrasoft X-ray irradiation.

After irradiation, each sample was recovered with 20 µl of 1xTE buffer. Two glycosylases, Endo III (Nth) and formamidopyrimidine DNA glycosylase (Fpg) were used as enzymatic probes. The enzymatic procedures and quantification of ssb, dsb and base damage were described previously [3].

Results and Discussion

The dependence of the amount of closed circular and linear DNA on dose is shown in Figure 1 for irradiation of DNA samples at 97% humidity at or around the phosphorus K-resonance energy. The main findings of this study are summarized as follows:-

1) 2.1 keV X-rays induce significant amounts of base lesions, the ratio of the yield of base lesions is about 50-60 % of that for prompt ssb, irrespective of the photon energy.

2) The yields of enzymatically induced dsb (clustered damage) significantly depend on the photon energy. With Fpg, the yields of additional dsb, relative to that for 2147 eV, are 1 and 1:2 for 2153 and 2160 eV respectively.

These results indicate that the phosphorus K-ionization (not K-resonance) efficiently induces clustered damage.

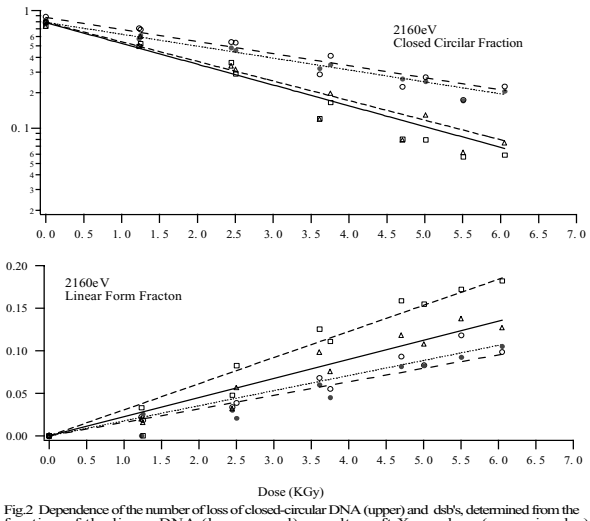

Fig.2 Dependence of the number of loss of closed-circular DNA (upper) and dsb's, determined from the fraction of the linear DNA (lower panel), on ultrasoft X-ray dose (open circular) or following a post irradiation incubated in the absense (closed circlar) or presense of either Nth (triangle) or Fpg (square).

References

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