Research Group for Radiation and Biomolecular Sciences

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The research objective of the research group is to fully characterize molecular processes by which ionizing radiation alters the chemical structure of DNA in cells. Two approaches were proposed to address the main objective: 1) Obtain experimental evidence of repairability of artificial clustered DNA damage *in vitro*. 2) Determine the yields of DNA lesions in a simple model DNA molecule, in terms of LET (Linear Energy Transfer) as an index of charged particle-radiation.

***In vitro* study of repairability of 8-oxoG/single strand break-containing clusters depends on their relative positions**

The biological consequences of clusters DNA damage site containing a single strand break (SSB) and nucleobase lesion(s) remain largely unknown. We examined the repairbility of two- and three-lesion clustered damage sites containing a 1-nucleotide gap (GAP) and 8-oxo-7,8-dihydroguanine(s) (8-oxoG(s)) by a base excision repair enzyme, Fpg, *in vitro* [1]. Fpg specifically excises purine base lesions such as 8-oxoG residues from DNA backbone via its AP lyase activity, and subsequently cleaves the DNA. The processing of the tandem cluster by Fpg leads to the generation of an additional band with a slightly larger size (18 mer OH termini) than that observed after excision of a single 8-oxoG (Fig. 1).

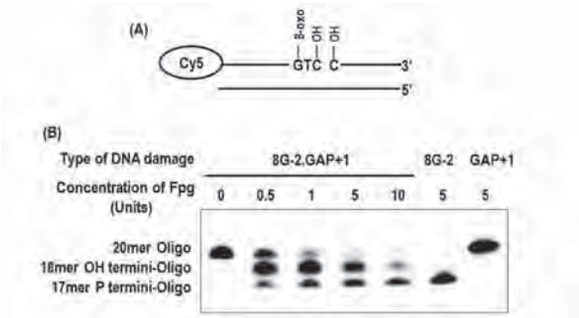

Fig. 1 Gel image of the excision of an 8-oxoG by Fpg placed in tandem with a GAP. (A) The shematic diagram of a Cy5-labeled double stranded oligonucleotide containing a GAP and an 8-oxoG tamdem clsuter. (B) 5' Cy5-labeled tandem cluster (8G-2, GAP+1) was incubated with increasing amounts (0-10 units) of Fpg. The configuration of the tandem cluster is indicated above the gel. 5' Cy5-labeled duplex oligonucleotides containing a single 8-oxoG (8G-2) incubated with Fpg and a single GAP (GAP+1) are shown for comparison.

The activity of the enzyme causes successive β- and δ-elimination reactions and generates a 1-nt gap. We treated the cluster with 0.1M NaOH, as an alkaline treatment is known to convert the β-elimination product to δ-elimination product at the 3' end. The result that the additional band disappeared after the alkaline treatment indicates that the band corresponds to a fragment generated by β-elimination. Further, to look at the effect of an additional 8-oxoG on the opposite strand for the excision of the 8-oxoG placed with a GAP in tandem, a three-lesion cluster (two 8-oxoGs and one GAP) was treated with Fpg. The 8-oxoG in tandem with a GAP in the three-lesion cluster is excised in a fashion similar to the excision of the 8-oxoG with a GAP in the tandem two-lesion cluster, forming two bands. This indicates that the additional 8-oxoG on the complementary strand does not inhibit the action of Fpg at the 8-oxoG placed with a GAP in tandem.

LET dependence of the yield of DNA damage induced in fully hydrated DNA films by ion particle-irradiation

In order to clarify the characteristics of DNA damage induced by high LET charged particle-radiation, the yield of DNA damage induced in closed-circular plasmid DNA (pUC18) were measured after exposing to various kinds of radiation (He, Ne and C ions; 2 to 900 keV/μm) using the JAEA-TIARA and NIRS-HIMAC facilities [2]. Hydrated DNA samples were used together with base excision repair enzymes, Fpg (see above) and EndoIII, which mainly excises pyrimidine base lesions, to detect the lesions as enzyme sensitive sites (ESSs). The results show that 1) the yields of ESS decrease drastically with increasing LET and very few ESSs are induced >100 keV/μm, althoguh the yield of SSB does not depend significantly on LET of the ions (Fig. 2), 2) the yield of duble strand breaks (DSBs) increases with increasing LET, however, 3) those of clustered damage sites visualized as additional DSBs by enzymatic treatment decrease with increasing LET, and 4) C and Ne ions induce less nucleobase lesions than He ions for comparable LET values. These results indicate that the yields of clusters containing nucleobase lesions, which are less readily processed by the base excision repair enzymes, depend not only on LET but also on the ion particle used.

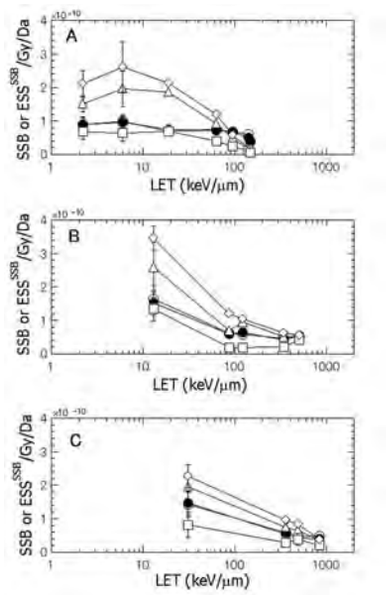

Fig. 2 Dependence of the yield of SSB or n(ESS or SSB) on LET for fully hydrated DNA irradiated with $^{4}He^{2+}$ ions (A), $^{12}C^{5+,6+}$ (B) and $^{20}Ne^{8+,10+}$ (C) at 5.6 °C (○), or following post-irradiation incubation for 30 min at 37 °C in the absence (●) or presence of either Nth (△), Fpg (□), or both Nth and Fpg (◇). The yields of isolated nucleobase lesions visualized by the enzymatic treatment decrease drastically with increasing LET.

References

[1] M. Noguchi *et al*., Mutat. Res. **732**, 34 (2012).
[2] T. Ushigome *et al*., Radiat. Res. **177**, 614 (2012).