X DNA

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                                              , University of Sherbrooke
               2008 1 2
                                 2008 2 21 1 , 2008 3 19 2 / 2008 3 21
Χ
                            DNA
                                                                        (Ta)
       pGEM-3Zf(-) plasmid DNA
                                                                   가 1.5 keVAI K X
                                      (monolayer)
                         DNA
10
                                                         (mean absorbed dose)
                                                      Χ
             3 eV
           plasmid DNA
                                (electrophoresis)
                                                         supercoiled DNAsupercoiled DNA
                       . Supercoiled DNA X eV8
                                                                             가
                       circular DNA crosslinked form 1 DNA
                                                                                           가
                                                       가
      supercoiled DNA가
                                                               (single strand break)
unsupercoiled DNA
                                                    Χ
                                                                                DNA
                   , DNA
                                                    (0~10 eV)
                                                                                 DNA
                 , X ,
    : DNA
1.
                                                          [3].
                                                                             2 가
                                                                              20 eV
                                                              2
   Χ
                              DNA
                                                가
                                                      1 MeV
                                                                               5×10 가
                                                                          DNA
      (single strand breaks: SSBs),
                                      가
                                              (double
                                                       (dissociative electron attachment: DEA)
strand breaks: DSBs)
                           DNA
                                                        . DEA
[1-3].
                       DNA
                                                                 가 DNA
                         DNA
           DNA
                                               , <sub>2</sub> DEA AB
                         (radical),
                 (secondary species)
                                     DNA
            [4-6]. 19
                                                           AB + e^{-} \rightarrow AB^{-(*)} \rightarrow A^{(*)} + B^{-(*)}
                                      DNA
                                                                       DNA
                                                         Sanche
                          2
                                         DNA가
                    가
                                                    plasmid DNA
                                                                      , oligonucleotide
          가
                                         Sanche
                       DNA
                                                  DNA
                         DNA
                                                                                   DNA
                                                                                 [7-14].
                                                                         DNA
                                                             Mark
    : , hcho@cnu.ac.kr,
                                                  DNA
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Χ
                                                                        가 1.5 keV
                                                                                     AI K
                          (fragments)
                           [4,15,16].
                                                         plasmid DNA
                                                                                                    (main
      가
                                                     chamber) plasmid DNA
                                                        (sample loading chamber)
100 eV
                                                                                      ~10<sup>-10</sup> torr
                      20 eV
                                                                                               24
                                                                                         ~10<sup>-9</sup> torr
                                                                                       (gate)
               Χ
                                      DNA
                                   2
            10 eV
                                                                                (differential pumping)
                                                                                       linear feedthrough가
                                   , X
plasmid DNA
                                                                     16
                                     plasmid DNA
                        가 DNA
                                                                        Χ
                                                                                              Χ
                                                                                  Keithley 610C solid-state
                                                     electrometer
2.
                                                     0.78 nA
                                                                         (flux) 2.49 × 10<sup>9</sup> electronscm<sup>-2</sup>s<sup>-1</sup>
   pGEM-3Zf(-) plasmid DNA(3197 bp.) collM109
                           QIAfilter Plasmid Giga [17-20].
Kit(Qiagen)
                                가
                      . 1%
                                                     plasmid DNA
              plasmid DNA 95% supercoiled DNA
                                                    plasmid DNA
5% unsupercoiled DNA
                                              . X (hatch)
                                                                 , plasmid DNA
                                        DNA
                                                                               linear feedthrough
                                                가
                                                     plasmid DNA
   Plasmid DNA
                                                      10
                                                                             (Faraday cup)
                가 0.025 mm
                                       Χ
                                                                            phosphor screen
       가 25×7 mm
                                                        phosphor screen
    가 14×14 mm
                                                            plasmid DNA ( = 5.1 \text{ mm})
                              μ115 TE
    plasmid DNA
                                        60 ng
plasmid DNA가
                                      plasmid DNA
                                                             1.4 nA
                                                                                          3 eV
                                                                           4.29 \times 10^{10} electronscm<sup>-2</sup>s<sup>-1</sup> .
                                     15<sub>1</sub>1 plasmid
                                                                                                    24
                                                                 10<sup>-10</sup> torr
DNA
                                         -40C
        . plasmid DNA
                                                                                      plasmid DNA
                                                                 , 7 , 10 3 XeV
       (sorption pump) 1~2
                                       plasmid DNA
                                                                              plasmid DNA
                                                                                               X
                 (monolayer)
5.1±0.2 mm
plasmid DNA
                                                                           (mean absorbed dollar)
                                       1.4 g<sup>2</sup>cm
 2 nm
                                                           (1)
                plasmid DNA
                                가 ~10<sup>-10</sup> torr
                                                                D_m = N_{tot} E_{ek} C_E / M_{DNA} \tag{1}
                 PHI Model 04-548 Dual Anode 2
Source(Perkin-Elmer)
                                                                                                , C_E
                                                                      1.6 × 10<sup>-19</sup> JeV<sup>-1</sup>
  4 kV, 1.1 mA
                                                                                         M_{DNA}
                                                                                              DNA
```

6 : DNA

> DNA 가

가

DNA,

70 **Gys** Gýs Χ 4.056 Χ 가  $ddH_2O$ 12:1 plasmid DNA DNA DNA 260 가 nm UV UV spectrophotometer(Hitachi U-2000) 가 가 DNA 98% DNA μľ (6×) 2 μl SYBR GREEN  $(100 \times)$ **15** . 1kb TAE mΜ DNA Marker DNA (40 가 1 mM EDTA, acetate, pH 8.0)가 1% 7 100 V, 68 75 V (450 STORM860 (Molecular Dynamics) Plasmid DNA ImageQuant (Molecular Dynamics)

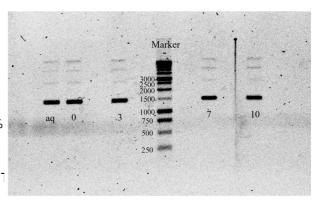
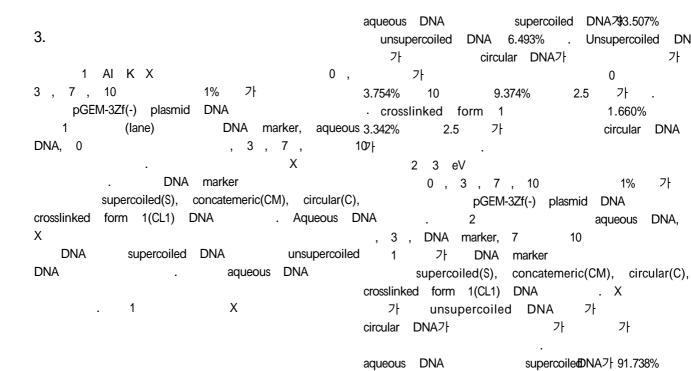


Fig. 2. Agarose gel electrophoresis of pGEM-3Zf(-) plasmid DNA irradiated by 3 eV electron beam for various times. The bands starting nm) from the top are crosslinked form 1, circular, concatemeric and supercoiled DNA, respectively.



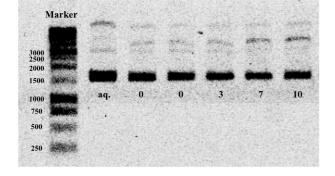


Fig. 1. Agarose gel electrophoresis of pGEM-3Zf(-) plasmid DNA irradiated by Al Ka X-rays for various times. The bands starting from the top are crosslinked form 1, circular, concatemeric and supercoiled DNA, respectively.

unsupercoiled DNA 8.262% Χ 2% Χ 93.500% 3 DNA **ImageQuant** DNA 5.0(Molecular dynamics) 3  $(a) \sim (c)$ Χ crosslinked form 1 DNA, supercoiled DNA, circular supercoiled DNA,  $(d)\sim(f)$ circular DNA 3 crosslinked form 1 DNA, (linear fitting) Χ DNA 98% Χ

6 : DNA

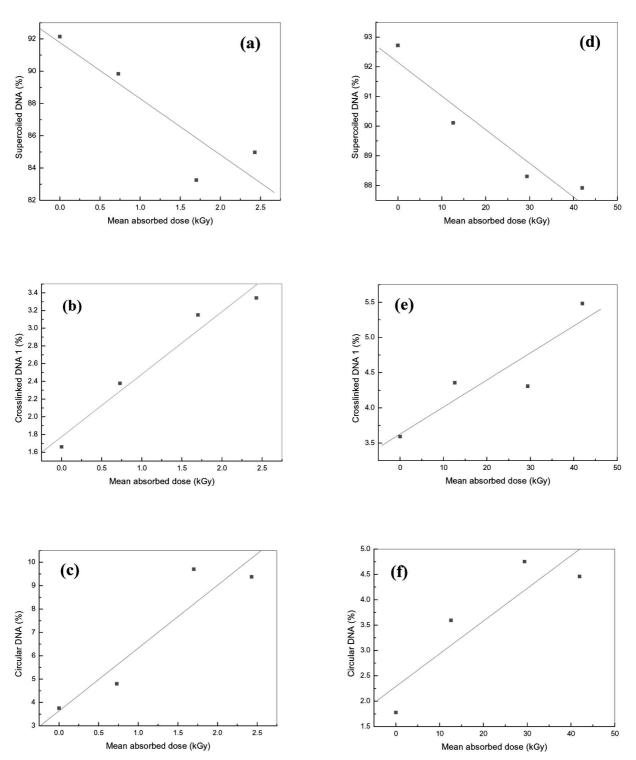


Fig. 3. X-ray exposure curves for the loss of (a) supercoiled as well as the formation of (b) crosslinked form 1, (c) circular DNA and electron beam exposure curves for the loss of (d) supercoiled as well as the formation of (e) crosslinked form 1, (f) circular DNA were irradiated in monolayer DNA film.

Table 1. The Specific Yields for Each DNA Form. S: supercoiled DNA, C: circular DNA, CL1: crosslinked form 1 DNA, CM: concatemeric DNA.

| 93.507%<br>1.190%<br>13.321% | 92.114%<br>3.754% | 89.838%<br>4.779% | 83.255%<br>9.670% | 84.975% |
|------------------------------|-------------------|-------------------|-------------------|---------|
|                              |                   | 4.779%            | 9.670%            |         |
| 13.321%                      |                   |                   | 7.07070           | 9.374%  |
|                              | 1.660%            | 2.378%            | 3.150%            | 3.342%  |
| 1.982%                       | 2.441%            | 2.985%            | 3.925%            | 2.309%  |
| 91.738%                      | 92.720%           | 90.112%           | 88.304%           | 87.919% |
| 2.463%                       | 1.776%            | 3.594%            | 4.753%            | 4.458%  |
| 13.198%                      | 3.592%            | 4.356%            | 4.307%            | 5.482%  |
| 2 (120/                      | 1.912%            | 1.928%            | 2.637%            | 2.141%  |
|                              | 13.198%<br>2.612% |                   |                   |         |

```
Zhongli
                           a7.⊦ X-ray
                    Caiet
                                        photoelectron
                                                                Χ
                                                                               DNA
spectrometer(XPS,
                    Perkin-Elmer)
                                                                         DNA
                                                                                      Χ
                                      eV
[17].
                                  1.4
(0~1486
         eV)
                                              5.8
                                                  eV,
                                                        10
eV
                                        3 eV
                                                                3
                                                                  eV
                                                                                                DNA
                                             DNA
          3
             eV
                                DNA
          3 eV
                                                                             가 DNA
                                                                                                           DNA
                                                                 3
                                                                    eV
                               Panajotoviet al. pGEM-
                             0.1~4.7 eV
                                           10
                                                             Χ
3Zf(-) plasmid
                DNA
                                               eV
                          가
                                                           가
                                                                       supercoiled
                                                                                     DNA
                 section: ECS)
                                                         unsupercoiled
                                                                        DNA
(effective cross
                                     [18].
                                                                                      supercoiled
                                가 DNA
                                                                  가
         (0.1)
                  4.7
                       eV)
                                                                                  . X
                                  가 3.1
                                                ECS
                                                            0 ~ 2.5 kGy
                                                                                           supercoiled DNA
                                                                                                                   9%
                                         eV
  = (12.3\pm1.0) \times 10^{-15} \text{ cm}^2
                                 , 1
                                       eV
  = (24.8\pm0.2) \times 10^{-15} cm<sup>2</sup>
                                      eV
                                                                40
                                                                     kGy
                                                                                        supercoiled
                                                                                                     DNA
                                                                                                                 5%
3.1 eV
                              ECS
                                        가
                                                                       Χ
                                                                                 (scale)
                                                                                           2.5 kGy
                                                        supercoiled DNA
                                                                                                                   3
eV
                                                         eV
                                                                         DNA
                                     1.4
                                         eV
                                                                     가
                                                                                                  Circular
                                                                                                           DNA
                                                                     DNA 1
     DNA
                  2
                                                         crosslinked
                         Zhongli
                                  0ati al
                                                Χ
                                                                 Χ
                                                                                            Circular DNA
                                                                                                                가
                       DNA
                                                                                          3
                                                                                             (c),
                                                                                                  (f)
                                              가 DNA
                                                                                                              5%
                                                                3
                                                                                           DNA
                                                                   eV
                                                                                   circular
                                                 [17].
       Panajotoviet
                    al.
                                                                                                    DNA
                    가
                                        가
                                             ECS
                                                                                                  DNA
                                                                        3
                                                                          eV
                DNA
                                         DNA
                            %가 circular
          %가 linear DNA
                      [18].
                                                                   DNA 가
                    DNA
                                          Χ
                                                                                DEA
                DNA
                                                                                 가
                                                                     [2,5].
                                                                                                       Barrieds al.
                                                                       10 eV
                                                                                                            DNA
                                     가
                                                                                                        DNA
                                                   eV
                                                3
                                                                               eV
                                                                                                          \pi^*
```

|   |                 | (tempor          | ary neg            |                       | ion; T                           | N  |
|---|-----------------|------------------|--------------------|-----------------------|----------------------------------|----|
| C-O<br>3 eV<br>10 eV  | ,<br>가          |                  | (anion)<br>가 DNA   | C                     | DNA                              | X  |
| ,<br>DNA  |                 |                  |                    |                       |                                  |    |
| ,<br>가<br>DEA   |                 | 가<br>, DNA<br>DN |                    | I                     | DNA<br>(suga                     | r) |
| 9 eV<br>tetrahydrofura<br>trapping cross<br>フト<br>cm <sup>2</sup> . |                 |                  | 1 e\               | TH                    | harge<br>HF<br>33×1 <sup>®</sup> |    |
| 4.  |                 |                  |                    | ~ OFM                 | 27()                             |    |
| plasmid DNA<br>keV) 0 , 3 ,<br>DNA<br>가 가                           | 7,              |                  | oiled D            |                       | ray (1.                          |    |
| form 1 DNA<br>supercoiled DNA<br>Supercoiled DNA                    | 4               | unsupe<br>가      | rcoiled<br>X       | ar, cro               | osslinke                         | d  |
| 3 eV<br>DNA 가   | 가<br>X          | (10 et           | / )                | , 3                   | eV                               |    |
| DNA   |                 | eV<br>eV         |                    |                       | フ                                | 'ŀ |
| フト<br>DEA<br>$\pi^*$<br>(anion)<br>$\pi^*$                          | TNI<br>-<br>C-C | C-O              | DNA                | DNA<br>A<br>DNA<br>π* |                                  |    |
|   |                 |                  | D<br>NA<br>-10 eV) | NA                    | DN                               | A  |

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DNA

DNA

(2006)

(DNA

TNI)

Χ

0~20

complex)

eV

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## DNA Damage by X-ray and Low Energy Electron Beam Irradiation

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Abstract - We observed DNA damages as a function of mean absorbed dose to identify the indirect effect of high-energy radiation such as x-ray. Monolayer films of lyophilized pGEM-3Zf(-) plasmid DNA deposited on tantalum foils were exposed to Al K X-ray (1.5 keV) for 0, 3, 7 and 10 min, respectively, in a condition of ultrahigh vacuum state. We compared DNA damages by X-ray irradiation with those by 3 eV electron irradiation. X-ray photons produced low-energy electrons (mainly below 20 eV) from the tantalum foils and DNA damage was induced chiefly by these electrons. For electron beam irradiation, DNA damage was directly caused by 3 eV electrons. Irradiated DNA was analyzed by agarose gel electrophoresis and quantified by ImagaQuant program. The quantities of remained supercoiled DNA after irradiation were linearly decreased as a function of mean absorbed dose. On the other hand, the yields of nicked circular (single strand break, SSB) and interduplex crosslinked form 1 DNA were linearly increased as a function of mean absorbed dose. From this study, it was confirmed that DNA damage was also induced by low energy electrons (0~10 eV) even below threshold energies for the ionization of DNA.

Keywords: DNA damage, Indirect action, X-ray, Low-energy electron beam, Dissociative electron attachment.