LET Dependence of DNA Single-Strand Scission in
E. coli B sub 1 by Charged Particles

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Log-phase cells of E. coli B sub 1, labelled with 3H-thymidine were bombarded with α-particles, carbon-
and nitrogen-ions of various energies and the LET dependence of the efficiency of DNA single-strand
break production (E sub b) was investigated. The amounts of radiation energy required for single-strand
break production proportionally increased with increasing LET, in the range greater than about 90 keV·μm⁻¹.
The heavier particle gave an LET-E sub b curve shifted to the higher LET as compared with that for the lighter particle. The relationship between the restricted LET (LET sub r) and E sub b corrected for δ-rays was represented by the same linear regression curve for the above three kinds of
particles. Analysis of the high-LET data in correlation with survival radiosensitivity indicated that
the number of particles passed through the DNA strand per D sub r decreases with increasing LET, ap-
proaching to the unity and the number of single-strand breaks per D sub r produced by the track-core
effect decreases toward a value of about 3. The results suggest that the traversal of a heavy charged
particle of high LET through the DNA strand may produce a double-strand break, which would be
of major importance in cell inactivation at high LET, but single-strand breaks produced mainly by
δ-rays is not necessarily lethal.

INTRODUCTION

The DNA strand breakage in bacterial cells and other biological systems produced by low-LET radiations such as X- or γ-rays and electrons have been extensively investi-
gated since McGrath and Williams sub 3) and Kaplan sub 4) have developed an unique method for sedimentation analysis on DNA. In contrast, only few reports on LET dependence of DNA strand break production sub 3-7) have been presented so far. Neary et al. sub 3) described the result obtained with T 7 phage and its isolated DNA that a single-strand break is formed on average for each energy loss event of 60 eV and there is little increase in double-strand break production upon the unrestricted LET (LET sub r) in the range up to 100 keV·μm⁻¹. More recently they extended their determinations up to 255 keV·μm⁻¹ and reported that the number of single-strand breaks per unit dose progressively increased with increasing LET beyond 100 keV·μm⁻¹ and the ratio of single- to double-strand break is constant sub 7). In contrast, Christensen et al. sub 6) observed with φX 174 RF DNA that single-
strand break frequency decreases three-fold and the double-strand break production increases five-fold at the maximum, as the unrestricted LET increases up to 2400 keV·μm⁻¹. Thus there is significant difference in the LET dependence of DNA strand breakage in the studies reported on bacteriophage DNA. Since DNA strand breaks would be one of the important damages in DNA caused by ionizing radiation which are associat-
ed with bacterial inactivation, more detailed informations on LET dependence of DNA breakage are anticipated to elucidate the basic mechanism for the biological effects of high-LET radiation.
The present paper will describe the measurement of single-strand DNA breaks in E. coli B\textsubscript{4-1} cells irradiated with various charged particles of different LETs accelerated in the IPCR cyclotron as well as 60Co γ-rays and the analysis on correlations of the DNA-strand break with cellular radiosensitivity.

MATERIALS AND METHODS

Log-phase cells E. coli B\textsubscript{4-1} grown at 37°C in a glucose-inorganic salt medium (2 g NH\textsubscript{4}Cl, 15 g Na\textsubscript{2}HPO\textsubscript{4}.12 H\textsubscript{2}O, 3 g KH\textsubscript{2}PO\textsubscript{4}, 3 g NaCl, 0.25 g MgSO\textsubscript{4}.7 H\textsubscript{2}O, 0.01 g CaCl\textsubscript{2}, 0.054 mg FeCl\textsubscript{3} and 2 g glucose per litre; pH 7.0) containing 20 µCi/ml 3H-thymidine were harvested, washed twice with 0.067 M phosphate buffer containing 0.01 M EDTA and resuspended in the same buffer at a concentration of about 10\textsuperscript{6} cells/ml. Each 0.05~0.1 ml aliquot of the cell suspension was fed on a membrane filter (Millipore filter type GS, 8 mm in diameter) which had been placed on a paper pad mounted on a sample wheel. The paper pad was made wet with 0.067 M phosphate buffer containing 0.01 M EDTA in advance. The monolayer of bacterial cells on the membrane filter obtained in this way was exposed in air at room temperature (20°C) to α-particles and heavy ions of carbon and nitrogen accelerated in the IPCR cyclotron of the variable energy type\textsuperscript{59}. The energy of cyclotron beams was varied over a range of 4~12 MeV/amu for α-particles and 4~8 MeV/amu for heavy ions. Fine adjustment of the beam energy at the bacterial sample was made by changing the thickness of air layer between the vacuum window and the bacterial sample as well as by the uses of the aluminum absorber foil. Beam energy and LET values were estimated by using tables given by Northcliffe and Schilling\textsuperscript{9}. For achieving the dose uniformity, membrane filters loaded with bacterial cells were swung in a plane perpendicular to the cyclotron beam during the bombardment. Dosimetry was performed by the nuclear electronic method\textsuperscript{10}. Immediately after irradiation, bacterial cells were resuspended in chilled 0.01 M Tris buffer containing 0.01 M EDTA and subjected to the sedimentation analysis of DNA. After the treatment with 0.13 mg/ml lysozyme at 37°C for 10 minutes, cells were lysed with 0.5 N NaOH on a top of the 5~20% alkaline sucrose density gradient (pH 12.5). Centrifugation was performed at 30,000 rev/min at 20°C for 100 minutes in a RPS 40-2 rotor in a Hitachi model 65-P ultracentrifuge. Then 10-drop fractions were collected on filter paper disks, followed by the determination of TCA-insoluble 3H radioactivity. Using a molecular-weight marker of T4 phage DNA, the number-average molecular weight of DNA (M\textsubscript{n}) was estimated from each sedimentation profile after the correction for the size of the drop on the determination of sedimentation distance. The number of single-strand breaks per gram of DNA (B) was calculated from M\textsubscript{n} values of DNA by an equation, B = N/M\textsubscript{n} (I) − N/M\textsubscript{n} (U), where M\textsubscript{n} (I) and M\textsubscript{n} (U) are the number-average molecular weight of DNA from irradiated and unirradiated cells, respectively, and N is Avogadro’s number. For each energy of different particles, determinations of DNA sedimentation profiles were made with three different doses in order to confirm a linear dose response of production of single-strand breaks. From the slope of the linear dose response curve, the efficiency of single-strand break production (E\textsubscript{sb} in eV/single break) was calculated. To examine the correlation of DNA-strand breakage with cellular radiosensitivity, the visible colony forming ability was also determined using meat extract-pepton agar plates.
RESULTS AND DISCUSSION

Some examples of the sedimentation profiles of \textit{E. coli} B\textsubscript{s-1} DNA obtained with different particles are illustrated in Fig. 1. The $E_{ab}$ values calculated for $\alpha$-particles and heavy ions of carbon and nitrogen having different LETs were found to increase with increasing unrestricted LET as shown in Fig. 2. Such LET dependence of $E_{ab}$ reveals the possibility that the appreciable amount of the beam energy might be wasted on the production of single-strand breaks in such a high LET region ($\text{LET}_{\text{a}} \gtrsim 90 \text{ keV} \cdot \text{µm}^{-1}$) as compared with that of X- or $\gamma$-rays.

Figure 2 indicates that these charged particles give three different linear lines representing the relationship between the unrestricted LET and $E_{ab}$, and a curve for the heavier particle shifts to the higher LET. This fact means that $E_{ab}$ values vary with different kinds of ions even at the same unrestricted LET. However, if one can assume that the production of DNA-strand breaks at the intersection of track-core and DNA strand is independent of each other in the region of sufficiently high LET, where the appreciable amount of absorbed energy is wasted on the production of strand breaks, it is expected that the relationship between the restricted LET and $E_{ab}$ can be represented by the same straight line over the wide range of the restricted LET after the appropriate correction for the $\delta$-ray effect.

\begin{figure}[h]
\centering
\begin{subfigure}{0.45\textwidth}
\centering
\includegraphics[width=\textwidth]{fig1a}
\caption{Fraction number}
\end{subfigure}
\begin{subfigure}{0.45\textwidth}
\centering
\includegraphics[width=\textwidth]{fig1b}
\caption{Percentage of total counts}
\end{subfigure}
\caption{Sedimentation profiles of $^3$H-DNA from \textit{E. coli} B\textsubscript{s-1}.}
\end{figure}

Bombarding particles and doses:

<table>
<thead>
<tr>
<th>Particle Type</th>
<th>LET (keV/µm)</th>
<th>61.7 krad</th>
<th>93.1 krad</th>
<th>123 krad</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$-particles (0.628 MeV/amu)</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td></td>
</tr>
<tr>
<td>C-ions (2.39 MeV/amu)</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td></td>
</tr>
<tr>
<td>N-ions (1.73 MeV/amu)</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td></td>
</tr>
<tr>
<td>$^{60}$Co $\gamma$-rays</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td></td>
</tr>
</tbody>
</table>
HEAVY IONS AND DNA SCISSIONS

A trial of the δ-ray effect correction for the data indicated in Fig. 3 A was made on the following assumption: (1) the rate of single-strand breakage produced by δ-rays is identical with that by 60Co γ-rays (E_{ab} = 40 eV/single break) as shown in Fig. 1; (2) a cut-off energy of 500 eV is employed to separate the δ-ray effect from the track-core effect; (3) the mean particle traversal length through the DNA is 3.3 nm, which is estimated by dividing the DNA volume of 3.66×10^{-3} μm^3 per cell by the geometrical cross section (S_g) of 1.1 μm^2.*

As the result of the above calculation, a linear relationship between E_{ab} corrected for δ-rays and the LET_{500} for α-particles and heavy ions of carbon and nitrogen was obtained over the wide range of restricted LET (Fig. 3 A).

The LET dependence of E_{ab} values found in the present study is similar to the result described by Christensen et al.* However, the efficiency of the single-strand break production observed here was considerably higher than that reported by Christensen et al. at the corresponding unrestricted LET, perhaps being due to different biological systems and the experimental methods. For 60Co γ-rays, E_{ab} calculated from the data reported by Christensen et al. (0.34 breaks/Mrad/10^4 daltons) is 305 eV/single break. According to the recent calculation by Town et al. 12) the true rate of strand breakage caused by X-rays in E. coli K-12 is ~52 eV/single break. We obtained the E_{ab} value of 40 eV/single break for 60Co γ-rays with E. coli B_{4,1}. Referring to these values obtained with E. coli at low-LET, the result indicated in Fig. 3 can be interpreted by a view that the waste of radiation energy for the production of single-strand breaks occurs in the LET_{500} region greater than 50 keV·μm^{-1} and the energy required for producing a single-strand break increases in a linear relation with increasing LET_{500}. On the other hand, the decrease in cellular radiosensitivity of E. coli B_{4,1} was observed from a little lower LET_{500} (10~30 keV·μm^{-1}) 8,13).

The LET dependence reported by Neary et al.* appears to be quite different from the results obtained by Christensen et al.* and the present study. Neary et al. de-

* For log-phase cells of E. coli B_{4,1}, the DNA content has been determined according to the method of Cooper and Helmstetter 11) to be 6.26×10^{-12}g/cell, which corresponds to a genome content of 1.5. Thus the DNA volume is 3.66×10^{-3} μm^3 per cell. The average projected area of the cellular nuclear region was estimated to be 1.1 μm^2 from the electron micrographs, which was referred to as the geometrical cross section (S_g) of the DNA target.
scribed that mean numbers of both single- and double-strand breaks per DNA molecule per unit dose increased as the unrestricted LET increased and the ratio of two types of breaks did not depend upon the unrestricted LET. Although both results described by Neary et al. and this work were obtained for track-segment irradiation, the irradiated materials used were different, that is, thin films of T7 phage DNA in the former and *E. coli* cells in the latter. In the comparison of these results, it should be taken in mind that different irradiation conditions for DNA molecules (*in vitro* and *in vivo*) were employed, which might give different spatial arrangement of DNA molecules. Another factor to be considered would be the presence of NaCl in DNA films prepared by Neary et al. In connection with the radiosensitization of bacterial cells by alkali halide, it was found that the presence of NaCl during gamma irradiation increased the number of single-strand breaks (unpublished data).

The number of single-strand breaks produced by the track-core effect of a given particle passing through DNA (*B*<sub>1</sub>) was calculated for the mean particle traversal length of 5.3 nm. Using a cut-off energy of 500 eV for the \( \delta \)-ray correction, the amounts of energy absorbed in DNA on a single particle traversal, which are given by \((\text{LET}_\infty \text{ of a given particle}) \times \text{(mean particle traversal length)}\), are partitioned into two portions afforded by the track-core and \( \delta \)-rays, respectively. The total number of single breaks can be determined from the empirical E<sub>b</sub> value and the amounts of energy absorbed in DNA on a single particle traversal. The number of single breaks produced by \( \delta \)-rays is obtained by dividing the amounts of energy in eV from \( \delta \)-rays by 40 eV required for single-strand break production by \(^{60}\text{Co} \gamma\)-rays. The difference between these two quantities is regarded as *B*<sub>1</sub>. As can be seen in Fig. 3B, *B*<sub>1</sub> values calculated in this way appear to be about 3 over the whole range of \( \text{LET}_{500} \) from 65 keV·\( \mu \)m\(^{-1}\) to 1500 keV·\( \mu \)m\(^{-1}\) (in DNA), being almost independent of the kind and LET of bombarding particles. Considering *B*<sub>1</sub> values of about 3 in high LET region as well as the short particle traversal length of 3.3 nm, it is likely that the DNA strand is distributed as a mono- or at the most double-layer on the average in *E. coli* *B*<sub>1</sub>-1 cells.

In order to examine the correlations of the DNA single-strand breakage with cellular radiosensitivity, the number of DNA single-strand breaks per *D*<sub>37</sub>, denoted by *B*<sub>37</sub>, was also estimated by dividing the amounts of energy absorbed in DNA by E<sub>b</sub> for the given particle. Similarly to the calculation deriving *B*<sub>1</sub> values mentioned above, a *B*<sub>37</sub> value is partitioned into *B*<sub>37</sub> and *B*<sub>\delta</sub> which are the number of breaks produced by track-core and \( \delta \)-ray effects, respectively, where *B*<sub>37</sub> = *B*<sub>\delta</sub> + *B*<sub>37</sub>. The values of *B*<sub>\delta</sub> decrease with increasing LET, approaching a value of about 3. In contrast, *B*<sub>37</sub> increases with increase of charge number of particles and mounts to the appreciable value several times higher than *B*<sub>\delta</sub> in the LET region for heavy ions. However, *B*<sub>37</sub> values for charged particles of the same kind do not appear to be significantly affected by the change in their LET. The considerable large number of *B*<sub>37</sub> determined for charged particles, especially heavy ions, suggests that single-strand breaks do not account for the loss of cell viability.

The mean number of particles passed through DNA per *D*<sub>37</sub> (*P*<sub>37</sub>) was calculated by:

\[
P_{37} = \frac{D_{37} \times \text{(DNA mass per cell)}}{(\text{LET}_\infty) \times \text{(mean particle traversal length)}}
\]

As shown in Table 1, *P*<sub>37</sub> values approach to the unity in the LET region of heavy
Table 1. Numbers of DNA single-strand breaks in E. coli B<sub>s-1</sub> and related values calculated for cyclotron beams

<table>
<thead>
<tr>
<th>Beam energy (MeV/amu)</th>
<th>α-particles</th>
<th>C-ions</th>
<th>N-ions</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.00</td>
<td>1.35</td>
<td>0.972</td>
<td>0.720</td>
</tr>
<tr>
<td>Unrestricted LET in DNA (keV/μm)</td>
<td>87.6</td>
<td>160</td>
<td>212</td>
</tr>
<tr>
<td>D&lt;sub&gt;57&lt;/sub&gt; (krad)</td>
<td>2.04</td>
<td>3.09</td>
<td>4.00</td>
</tr>
<tr>
<td>B&lt;sub&gt;57&lt;/sub&gt;</td>
<td>16.9</td>
<td>14.5</td>
<td>15.5</td>
</tr>
<tr>
<td>B&lt;sub&gt;37&lt;/sub&gt;</td>
<td>11.4</td>
<td>7.94</td>
<td>8.47</td>
</tr>
<tr>
<td>B&lt;sub&gt;37&lt;/sub&gt;</td>
<td>5.50</td>
<td>6.56</td>
<td>7.03</td>
</tr>
<tr>
<td>P&lt;sub&gt;37&lt;/sub&gt;</td>
<td>2.75</td>
<td>2.28</td>
<td>2.22</td>
</tr>
<tr>
<td>S&lt;sub&gt;x&lt;/sub&gt;/P&lt;sub&gt;37&lt;/sub&gt;</td>
<td>0.400</td>
<td>0.482</td>
<td>0.495</td>
</tr>
<tr>
<td>S&lt;sub&gt;x&lt;/sub&gt;/S&lt;sub&gt;eff&lt;/sub&gt;</td>
<td>0.412</td>
<td>0.487</td>
<td>0.500</td>
</tr>
</tbody>
</table>

ions, although they are about 2 for low-energy α-particles. The decline of P<sub>37</sub> values in high-LET region toward the unity suggests that on the traversal of a single heavy ion through DNA, at least one lethal event is always produced, but it is not the case on the α-particle traversal. In Table 1, it should be noted the values of S<sub>x</sub>/P<sub>37</sub> for α-particles are consistent with those of the effective inactivation cross section (S<sub>eff</sub>) determined in our separate experiment. This agreement suggests that the choice of parameter values used in the δ-ray correction has been appropriate. On the other hand, the values of S<sub>x</sub>/P<sub>37</sub> for heavy ions appear to be somewhat greater than S<sub>eff</sub>. This difference could be explained by assuming some contribution of δ-rays emerged from heavy ion tracks to the cell inactivation perhaps by their overlapping effect. Since DNA double-strand breakage is considered to be irrepairable in E. coli B<sub>s-1</sub> to cause cell death, the results of the present analysis on DNA single-strand break production lead us to a view that at very high LET, lethal damages in E. coli B<sub>s-1</sub> cells are caused mainly by the traversal of heavy ions through the DNA strand, which may result in the formation of double-strand breakage. There would be also the possibility that the cell inactivation is caused partly by the cumulative effect of overlapping δ-rays from heavy ions, which may also afford the double-strand break.

According to Freifelder<sup>16</sup>, there are two principal types of lethal damages in X-irradiated double-strand DNA phage, that is, (a) DNA double-strand breaks which are O<sub>2</sub>-independent and occur at ca. one-half the rate of production of lethal hits and (b) O<sub>2</sub>-dependent damages which are thought to be the alteration of DNA bases. Taking account of the observation that the inactivation of living cells by charged particles is O<sub>2</sub>-independent<sup>17</sup>, DNA double-strand breaks induced by the charged particle-traversal might be regarded as the decisive event for the cell inactivation at high LET.

ACKNOWLEDGEMENT

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Appendix

Determination of Cut-off Energy for $\delta$-rays, $\omega_0$

Since several methods$^{18-20}$ have been presented to estimate high-energy $\delta$-ray contribution on the inactivation of bacterial cells by heavy charged particles, we attempted to apply these methods to the $\delta$-ray correction for the estimation of track-core effect on DNA single-strand breakage. A method employed in our calculation to separate the effect of $\delta$-rays from that of the track core is based on the theory developed by Dolphin-Hutchinson$^{18}$. The fraction of the total energy delivered by $\delta$-electrons (f) was given by the following relations:

$$f = \frac{L_{\omega>\omega_0}}{L_\omega} = \frac{L_{\omega>\omega_0}}{L_{\omega<\omega_0} + L_{\omega>\omega_0}}$$

where $L_\omega$ is the unrestricted LET, $L_{\omega<\omega_0}$ is the restricted LET and $L_{\omega>\omega_0}$ refers to the energy loss by $\delta$-electrons ejected from the track with energy greater than $\omega_0$.

On the assumption that the efficiency of the DNA single-strand break production by $\delta$-rays is consistent with that of $^{60}$Co $\gamma$-rays (40 eV/sb), corrected $E_{sb}$ was estimated by the equation as follows:

$$\text{Corrected } E_{sb} = \frac{E}{E/E_{sb} - f(E/40)} = \frac{1}{1/E_{sb} - f/40}$$

where E in eV is defined as an energy absorbed in the DNA molecule of bacterial cell, $E/E_{sb}$ is the number of total breaks produced in DNA and $f(E/40)$ is the number of breaks produced by $\delta$-rays.

In order to get a linear relationship between the corrected $E_{sb}$ and the restricted LET, the selection of cut-off energy $\omega_0$ was examined over the range of 200$\sim$1000 eV. As the result of this examination, the value of 500 eV was employed as a cut-off energy for $\delta$-ray correction.

REFERENCES


