A Model for the Induction of DNA Damages by Fast Neutrons and their Evolution into Cell Clonogenic Inactivation

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Model/Radiation mechanism/Inactivation cross section/DNA damage/Neutron.

It has been long stated that cellular inactivation through neutron irradiation is mainly caused by energy deposition in DNA molecules from recoiled secondary charged particles. Complexities associated with neutrons, such as the generally broad energy spectrum and the inherently wide energy spectrum of the induced charged particles, not to mention that the dependence of cellular inactivation by charged particles on radiation quality is yet to be fully understood, make it difficult to check this statement. Recently a molecular model has been proposed that improves the quantitative explanation of the dependence of cellular inactivation by charged particles on radiation quality. An attempt was made to apply this model for analysis of neutron cellular inactivation. As a preliminary result it is suggested that neutron cellular inactivation is caused not only by secondary charged particles but also by an “atomic deletion” effect, generated by a stripped atom recoiling from a DNA molecule. This effect seems to be of significant importance, the inactivation cross section of this effect for fission neutrons is as much as 15% (aerobic conditions) or 55% (hypoxic) of the total, and the severity of one occurrence of atomic deletion by a single neutron is estimated as much as 3.1 \( \pm \) 1.1 times (aerobic) or 6.8 \( \pm \) 1.2 times (hypoxic) higher than the severity of one event by a single track of a charged particle interacting with DNA.

INTRODUCTION

Neutron irradiation of living cells leads to the induction of the same biological endpoints, such as cell death, chromosome aberrations, mutations, cancer, as gamma or X radiation and yet the dose effect relationship following neutron irradiation tends to be “straighter” or more linear when displayed in the usual semi-logarithmic plot than following gamma irradiation and the biological effect is not influenced to the same extent by changes in the irradiation conditions.\(^1\) These differences have been previously explained by assuming that neutrons cause more ‘direct effects’ and less ‘indirect effects’ or that neutrons cause less ‘physiological’ damage, such as changes in the surface properties of chromosomes and more ‘genetic’ damage, such as mutations’ or more ‘structural’ damage, such as chromosome aberrations.\(^1\)–\(^3\)

Biological material absorbs energy from fast neutrons through the action of secondary charged particles, mostly protons and to a smaller extent heavy recoils (partially stripped nuclei of C, N, and O) and products of nuclear reactions such as \( \alpha \) particles. In the case of neutrons with energy between 5 and 20 MeV recoil protons have ranges up to a few millimeters, while the \( \alpha \) particles have a range from 10 to 100 micrometers and heavy recoils a few micrometers.\(^4\)

Since neutrons dissipate their energy in tissue through different interactions with the various constituents of the material, the energy deposition is characterized by a complex linear energy transfer (LET) spectrum. Therefore neutrons are less suited for fundamental investigations of the mechanisms by which effects of ionizing radiation on living cells are initiated.\(^5\) Studies of the relative biological effectiveness (RBE) of directly ionizing particles with widely differing LET can provide information on mechanisms about the dimensions of the critical structures involved in the initiation of radiation damage in cells, and about the number of ionizations required in that structure for the production of the considered biological endpoints.\(^6\)–\(^11\) Based on these data many phenomenological and mechanistic models have been proposed\(^12\)\(^{,13}\) to explain the dependence of cellular radiosensitivity on radiation-quality. It is, however, the current status of theoretical radiation biology that no studies have yet shown a single or unique set of descriptive variables sufficient for understanding radiosensitivity and its dependence on radiation-quality. LET cannot be a unique variable quantifying radiation-quality from the fact that observed radiosensitivities are different between two charged particles.
with a different charge and velocity having the same LET.\textsuperscript{3)}

A mechanistic model has been recently proposed\textsuperscript{14)} in which the processes of radiation action for cell killing are separated into damage-induction and damage-repair and in which molecular DNA damages are connected to cellular damages. It was found that primary ionization mean free path is a unique variable of radiation-quality for estimates of initial DNA damages and it is also a unique descriptive variable for improved quantitative estimates of inactivation cross section of repair deficient AT (Ataxia telangectasia) cells for different radiation qualities. The model also provides quantitative estimates of inactivation cross sections for repair proficient cells to different radiation qualities. The purpose of this work is to attempt, based on this model, to answer the question whether or not cellular biological response after neutron irradiation is exclusively due to energy deposition in DNA from secondary heavy charged particles set in motion by neutron recoil.

\section*{MATERIALS AND METHODS}

\textbf{The model of cellular inactivation induced by charged particles\textsuperscript{14)}}

The inactivation cross section for charged particles is proportional to the probability of residual irreparable DNA damages. Initial DNA damage per single charged particle track is induced by any of 5 reaction modes of different possible combinations between direct (D) and indirect (W) actions (Fig. 1). The probability of occurrence of initial DNA damage in each reaction mode is calculated by target theory\textsuperscript{2)} applying a ‘hit-event’ between the primary ionization mean free path of the considered charged particle and a distance specific for each reaction mode. These specific target distances are defined in a molecular structure in which 10 base-pairs DNA is surrounded by water molecules of depth c (nm), that is, once c is determined, the sample average of relevant distances between atoms to that of a particular reaction mode is calculated. Three parameters of the model, c, \(\sigma_d\) and p (as described below), which relates initial DNA damages to cellular inactivation cross section, are optimized using data of repair deficient AT cells. The data of AT cells suggest two types of molecular structure concerning water depth, one fully cylindrical around DNA with a depth of 4.6–4.9 nm (structure B) and the other a half cylinder with a depth of 3.7–4.3 nm (structure C), which may correspond to parts of a nucleosome, namely, the linker DNA (27%) and the wrapped DNA (73%) around a nucleosome core, respectively. This finding is found to be important when possible secondary charged particles by neutrons are taken into consideration.

In addition to the model reproducing the data of AT cells, the model also suggests that the inactivation cross section for repair proficient cells such as T1 cells\textsuperscript{6,9)} and Hela cells\textsuperscript{15)} may be explained by combinations of reaction modes related preferentially to direct actions, such as D-D, D-W and D-W under aerobic conditions (Fig. 2) or D-D and D-W under hypoxic conditions (Fig. 3), which is the case in both DNA structures B and C. In Figs. 2 and 3 the inactivation cross sections \(\sigma_B(\lambda)\) and \(\sigma_C(\lambda)\) are shown, where \(\sigma_B(\lambda)\) and \(\sigma_C(\lambda)\) are those for structure B and C respectively and 0.27 and 0.73 are the fractions of the 54 bp of linker DNA and the 146 bp of wrapped DNA of the 200 bp associated with a nucleosome.\textsuperscript{16)} Since data on cell death induced by neutrons are limited for repair proficient cells, these suggestions of relevant reaction modes should be a guide to study the mechanisms of radiation action by neutrons.

Applying the model to neutrons consists of assumptions and approximations specific to neutrons as described below. 1. The inactivation cross section of repair proficient cells for a neutron is estimated by inactivation cross sections

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Fig_1.png}
\caption{Five modes of DNA damage by a single track of a charged particle across the molecular target. The target consists of 10 base-pairs of DNA surrounded by water molecules. \(\lambda\) is the primary ionization mean free path of the charged particle in liquid water. The symbols of star and circle show lesions on a DNA strand and OH radical production, respectively. Mode D-D can produce lethal damage consisting of two lesions (one in each strand) by direct action from a single track. Mode D-W or mode W-W can produce lethal damage through a direct action on one strand and an attack by an OH radical on the opposite strand or attacks by two OH radicals on opposite strands from a single track with an efficiency of \(p\) \((= 0.11)\) for an OH radical induced lesion. Mode D or mode W can produce lethal damage with an efficiency of \(p\) through a single direct action or a single attack by an OH radical from a single track. The efficiency \(q = 0.11\) of an OH radical causing a single strand break is estimated experimentally\textsuperscript{11)} and this value is assumed as the efficiency of an OH radial eventually causing cell death in the present model.}
\end{figure}
Fig. 2. Inactivation cross section for repair proficient cells under aerobic conditions as a function of the primary ionization mean free path $\lambda$. Experimental data: for T1-cells by Ar-, Ne- and C-ions, $^9$ O-ions, $^6$ and for Hela cells by H-ions $^15$ are plotted against estimated $\lambda$. The top dotted curve No. 5 termed ‘compo. 12435’ shows the inactivation cross section for AT-cells for charged particles, $^{14}$ meaning $\sigma(\lambda) = \sigma(D-D) + \sigma(D-W) + \sigma(D) + \sigma(W-W) + \sigma(W)$. Letters D-D, D-W, W-W, D and W indicate each contribution of the inactivation cross section and specified as number 1, 2, 3, 4 and 5 respectively. The number 1, 2, 3, 4 and 5 assigned to each curve indicate the cumulative inactivation cross section over the modes specified by the “compo” number. The solid curve (No.3 termed ‘compo. 124’) is partial inactivation cross section for AT cells for charged particles $^{14}$ meaning $\sigma(\lambda) = \sigma(D-D) + \sigma(D-W)$, which is assumed to be the inactivation cross sections $\sigma_{\text{irreparable}}(\lambda)$ for repair proficient cells irradiated by secondary charged particles induced by neutrons.

Fig. 3. Inactivation cross section for repair proficient cells by charged particles under hypoxic conditions as a function of the primary ionization mean free path $\lambda$. The experimental data are for T1 cells by Ar-, Ne- and C-ions. $^9$ The solid curve (No.2 termed ‘compo. 12’) is $\sigma(\lambda) = \sigma(D-D) + \sigma(D-W)$, which is assumed to be the inactivation cross sections $\sigma_{\text{irreparable}}(\lambda)$ for repair proficient cells irradiated by charged particles induced from neutron irradiation.

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$\sigma_{\text{irreparable}}(\lambda_j)$ for secondary charged particles induced in the molecular target by the neutron. Those inactivation cross sections are $\sigma_{\text{irreparable}}(\lambda_j) = \sigma(D-D) + \sigma(D-W) + \sigma(D)$ for aerobic condition (Fig. 2) or $\sigma_{\text{irreparable}}(\lambda_j) = \sigma(D-D) + \sigma(D-W)$ for hypoxic conditions (Fig. 3). It is noted that the estimated inactivation cross sections are slight overestimations for hydrogen - ions in Fig. 2 and for all ions in the Fig. 3, which slightly handicap the present study to answer the question proposed. This may suggest that the inactivation cross section for repair proficient cells cannot be explained simply by some combination of the production modes of initial damages and inactivation cross section for repair proficient cells should be explained by a model for repair processes in future.

2. Inactivation cross section of repair proficient cells $\sigma_{\text{irreparable}}(\lambda_j)$ is the same mathematical statement for both structure B and C, differences in the kinds of secondary charged particles generated between structure B and C is taken into consideration.

3. Almost all secondary charged particles are “starters”, which are induced inside the cell nucleus (or cell) by recoil from a neutron interaction, and irradiate part of the cell nucleus depending on their energy. This results in modifying the saturated inactivation cross section $\sigma_s$ to $\sigma_s = \sigma_s[1 - \exp(-\frac{R_j}{l_c})]$, where $\sigma_s$ is the saturated cross section observed with external irradiation of the charged particle. As the energy of a charged particle decreases, the experimental inactivation cross section increases and converges to a constant value called the saturated inactivation cross section. $R_j$ is the range and $l_c$ is the effective path length of the secondary charged particle. $l_c$ is regarded as a parameter specific to neutrons and is optimized by fitting the model to the data of cellular inactivation by neutrons.

4. A new process to cause cellular inactivation other than from secondary charged particles is introduced. It is the process that a neutron hits a DNA molecule and recoils any of the atoms that compose DNA, in this work it is termed “atomic deletion”. Possible occurrences of this are estimated in terms of the current molecular target (Fig. 4) as a combination of the expression $\sigma_{\text{irreparable}}(\lambda_j) = \sigma(D-D) + \sigma(D-W) + \sigma(D)$ of the charged particle model multiplied by a new parameter $\eta$ representing the efficiency of this process to the cellular effect. The modifications of the saturated inactivation cross sections (step 3) are not applied for these inactivation cross sections, since the significance of the phenomena is ‘all or nothing’ in nature. The chemical bond may be broken at a site of the atomic deletion, since the average displacement of bare H-, C-, N-, O- and P- atoms is estimated to be longer than 0.4 nm when recoiled by neutrons of energy higher than 0.001 keV, which is sufficient to break the chemical bonds.

5. The induction of DNA damage is modeled on the basis of comparisons between the primary ionization mean free path and the distance between pairs of ionized atoms, such distances being characteristic of the mode of radiation action and relative occurrences of the reaction modes. The number of OH radicals per average energy to produce an ion pair on

![Fig. 4. Schematic showing of the atomic deletion. Secondary heavy charged particles from neutron scattering DNA can be an hydrogen, carbon, nitrogen, oxygen or a phosphorous nucleus. These secondary heavy charged particles may cause modes D-D, D-W, or D, while D is here an atomic deletion (the first D in the mode D-D).](https://academic.oup.com/jrr/article-abstract/48/4/289/992573)
the nanosecond time scale is used for indirect action. Assuming a relationship between estimated yields of DNA damages and experimental inactivation cross sections for AT-cells, the present model enables a quantitative reproduction of experimental results for AT-cell killing under aerobic or hypoxic conditions. The inactivation cross section of repair deficient (AT) cells for a charged particle of primary mean free path \( \lambda \) is given as follows:

\[
\sigma(\lambda) = \sigma(D-D) + \sigma(D-W) + \sigma(W-W) + \sigma(D) + \sigma(W)
\]  

(1)

\[
= \sigma_s \left[ f_1 \left(1 - \exp\left(-\frac{t_{01}}{\lambda}\right)\right) + f_2 \left(1 - \exp\left(-\frac{t_{02}}{\lambda}\right)\right)^2 \right] 
\]

\[
+ f_3 \left(1 - \exp\left(-\frac{t_{03}}{\lambda}\right)\right)^2 + f_4 \left[1 - \exp\left(-\frac{t_{04}}{\lambda}\right)\right] 
\]

\[
+ f_5 \left[1 - \exp\left(-\frac{t_{05}}{\lambda}\right)\right]
\]  

(2)

where

\[
f_1 = \frac{n_1}{N}
\]

\[
f_2 = \frac{g(\lambda) q n_2}{N}
\]

\[
f_3 = \frac{g(\lambda^2) q^2 n_3}{N}
\]

\[
f_4 = \frac{p n_4}{N}
\]

\[
f_5 = \frac{p g(\lambda) q n_5}{N}
\]  

(3)

(4)

(5)

(6)

(7)

where \( \sigma_j \) is the geometrical cross section of cell nucleus, \( t_{0j} \) (j = 1, 2, 3, 4 and 5) the average distance and \( n_j \) (j = 1, 2, 3, 4 and 5) the frequencies of the modes of D-D, D-W, W-W, D, and W, respectively. \( q \) is the efficiency of an OH radical produced around DNA to eventually cause lethal damage in either strand of DNA (q = 0.11 is assumed), \( p \) is the efficiency of DNA damage induced by modes D or W causing a lethal event, \( g(\lambda) \) is the yield of OH per unit energy to produce an ionization, which is an estimation of the nanosecond time scale yields of OH, and \( N \) is the normalization constant under an approximation that the inactivation cross section approaches to a value for the minimum \( \lambda \) of each accelerated charged particles in liquid water, for example, that of \( \lambda \) for H-, C-, N-, O-, and P- ion is estimated to be 0.89 nm, 0.2 nm, 0.17 nm, 0.16 nm and 0.11 nm, respectively. For neutrons it is subject to modification (step 3) as follows,

\[
\sigma = \sigma_s[1 - \exp\left(-\frac{R_c}{L}\right)]
\]  

(9)

where, \( \sigma_s \) is the saturated inactivation cross section, \( R_c \) is the range of the charged particle and \( L \) is the effective path length of the charged particle inside the cell nucleus. When the energy of the charged particle is high enough as \( R_c >> L \), it holds \( \sigma_s = \sigma_s \) with \( L \) = mean chord length of cell nucleus.

6. For the case of structure B the inactivation cross section of repair proficient cells for neutrons of energy \( E \) consists of two components, atomic deletion component \( \sigma_{\text{deletion}} (E) \) and secondary charged particle component \( \sigma_{\text{charged}} (E) \).

\[
\sigma_{\text{total}} (E) = \eta \cdot \sigma_{\text{deletion}} (E) + \sigma_{\text{charged}} (E)
\]  

(10)

\[
\sigma_{\text{deletion}} (E) = \int_{E_{\text{f}}}^{E\text{max}} \sum_{j=H,C,N,O,P} h_j f_{j,\text{arg}} \sigma_{n,j} (E) \sigma_{\text{irreparable}} (\lambda_j) l
\]  

(11)

\[
\sigma_{\text{charged}} (E) = \int_{E_{\text{f}}}^{E\text{max}} \sum_{j=H,C,N,O,P} h_j f_{j,\text{arg}} \sigma_{n,j} (E) \frac{\sigma_{\text{irreparable}} (\lambda_j)}{l}
\]  

(12)

\[
\sigma_{\text{total}} (E) = \sigma_{\text{water}} (E) + \sigma_{\text{charged}} (E)
\]  

(13)

\[
\sigma_{\text{charged}} (E) = f_{\text{water}} \sum_{j=H,O} k_j \sigma_{n,j} (E) \frac{\sigma_{\text{irreparable}} (\lambda_j)}{l}
\]  

(14)

\[
\sum_{j=H,O} k_j = 1
\]  

(15)

\[
f_{\text{DNA}} + f_{\text{water}} = 1
\]  

(16)

\[
\lambda_j = \lambda (T_{1/2}) = \lambda \left( \frac{4 A_j}{(A_j + 1)^2} \right)
\]  

(17)

\[
\text{LET}_d (E) = \sum_{r=H,O} k_r \sigma_{n,r} (E) \frac{\text{LET}_d (T_{1/2})}{l}
\]  

(18)

\[
\sum_{r=H,O} k_r \sigma_{n,r} (E)
\]
0.346, $h_C = 0.299$, $h_N = 0.118$, $h_O = 0.205$ and $h_P = 0.032)$. $k_j$ is the fraction of j-th atom in water ($k_H = 0.667$ and $k_O = 0.333$). $\sigma_{n,j}(E)$ is the total reaction cross section by neutrons of energy $E$ for j-th atomic nucleus. It is assumed in this work that only elastic scattering occurs according to the value of this cross section. $\lambda_j$ is the primary ionization mean free path of j-th recoiled charged particle which is calculated by eq.(17) for its average recoiled energy. The parameter $\eta$ is critical, the optimum value of which is determined by comparison with experimental data. Eq.(18) gives the primary LET of neutrons and is used to calculate experimental inactivation cross section $\sigma_{\exp}(\mu m^2)$ from experimental radiosensitivity $\alpha$(Gy$^{-1}$) with $\sigma_{\exp}(\mu m^2) = 0.16 \times$ LET$_n$(E) $\times \alpha$(Gy$^{-1}$), where the survival fractions as a function of absorbed dose D(Gy) are well fit with the formulae exp(-$\alpha$D). For the case of structure C (Fig. 5) inactivation cross section of repair proficient cells for neutrons consists of two components, atomic deletion component $\sigma_{\text{deletion}}(E)$ and secondary charged particle component $\sigma_{\text{charged}}(E)$. There are two origins of secondary charged particles for the latter, water and nucleosome.

\[
\sigma_{\text{total}}(E) = \eta \sigma_{\text{deletion}}(E) + \sigma_{\text{charged}}(E)
\]

where $f_{\text{DNA}}$, $f_{\text{water}}$, and $f_{\text{protein}}$ are the fractions of atoms in DNA, water and nucleosome, respectively, where the numbers of atoms of DNA, water, and protein is 645, 7969, 7969 (aerobic) or 645, 9751, 9751 (hypoxic) respectively. $\sigma_{\text{protein}}(E)$ and $\sigma_{\text{water}}(E)$ are calculated by eqs.(11) and (14), respectively, $p_j$ is the fraction of atoms (H,C,N,O) of a nucleosome ($p_H = 0.027$, $p_C = 0.645$, $p_N = 0.157$ and $p_O = 0.171$), which is counted in a protein which ignores sulphur atoms. $\sigma_{n,j}(E)$ is the neutron reaction cross section, $\lambda_j$ is the primary ionization mean free path. The assumption of equal number of atoms in water and protein is simply an initial setup for the study and it is modified according to the optimization of the model with experimental data.

![Fig. 5](https://example.com)  
Secondary heavy charged particle recoil from surrounding molecules in the molecular target under neutron irradiation. The secondary charged particle can be an hydrogen or an oxygen nucleus from water, or an hydrogen, carbon, nitrogen and oxygen nucleus from the nucleosome core, when only elastic scatterings are considered. The number of atoms in both groupings is examined during optimization of the model parameters.
We have used data on the neutron irradiated inactivation of AT cells,\(^2\) T1 cells,\(^5\) C3H10T1/2 cells,\(^2\) Hela cells\(^3\) and P-388 cells.\(^4\)
The parameters of the model are \(\eta\) the efficiency of atomic deletion event and \(l_c\) the effective path length of the secondary charged particles. We search for minimization for the parameters
\[
\chi^2 = \sum \left[ \frac{\sigma_{exp}(E_i) - \left( \eta \sigma_{\text{deletion}}(E_i) + \sigma_{\text{ch arg}}(E_i) \right)}{\sigma_{exp}(E_i)} \right]^2 / \sigma_{exp}(E_i)
\]
and compare the minimum with values of \(\chi^2\) for other optimizations when \(\eta = 1\) that the atomic deletion is the same efficiency as the charged particles to cause lethal damages, or \(\eta = 0\) that no event of the atomic deletion exists at all.

**RESULTS**

**Cellular inactivation induced by neutrons**
Quantitative estimates of the number of irreparable lethal lesions induced by charged particles are seen to be those produced preferentially by direct reaction modes such as those of D-D, D-W and/or D (Figs. 2 and 3), which can be produced preferentially by direct reaction modes such as lesions induced by charged particles. We search for minimization for the parameters

Table 1. Optimized parameters of the present model with n data

|                     | Aerobic |                     | Hypoxic |
|---------------------|---------|---------------------|---------|
| Cell inactivation   |         |                     |         |
| B structure         | \(\eta\) | 3.1 ± 1.1           | 6.8 ± 1.2 |
| \(l_c\) (\(\mu\)m) | 1.8 ± 0.6 | 1.5 ± 0.4           | 2.4 ± 0.4 |
| \(\chi^2\)         | 46.9    | 51.4                | 24.3    |
| C structure         |         |                     |         |
| \(\eta\)           | 4.8 ± 1.1 | 1                   | 7.4 ± 1.8 |
| \(l_c\) (\(\mu\)m) | 2.9 ± 1.5 | 2.3 ± 1.0           | 5.1 ± 1.1 |
| \(\chi^2\)         | 60.3    | 61.7                | 25.5    |
| Cell transformation |         |                     |         |
| B structure         | \(\eta\) | 2.1 ± 1.6           | 8.9 ± 1.4 |
| \(l_c\) (\(\mu\)m) | 1.6 ± 0.9 | 1.0 ± 0.3           | 3.0 ± 0.5 |
| \(\chi^2\times 10^7\) | 0.98    | 1.14                | 1.12    |
| C structure         |         |                     |         |
| \(\eta\)           | 8.9 ± 1.4 | 1                   | 3.0 ± 0.5 |
| \(l_c\) (\(\mu\)m) | 3.0 ± 0.5 | 2.9 ± 0.4           | 2.9 ± 0.4 |
| \(\chi^2\times 10^7\) | 1.12    | 1.94                | 2.41    |

\(^{2}\) J. Radiat. Res., Vol. 48, No. 4 (2007); http://jrr.jstage.jst.go.jp
nucleus (the long dashed curve), respectively. The contribution from the recoiled hydrogen ion is dominant for the neutron energies $E_n < 1000$ keV, while that from the recoiled oxygen ion is dominant for the neutron energies $E_n > 1000$ keV. This result comes from the combinations of four factors for hydrogen and oxygen, namely, (1) neutron interaction cross section, (2) relative abundance in the medium within the target, (3) inactivation cross section $\sigma(\lambda)$ and (4) extent of the partial irradiation estimated by eq.(9). As neutron energy increases (a) from 10 keV to 100 keV, it reaches a peak due to competition of the factor (4) and the factor (3), and (c) for 200 keV and higher, it decreases mainly due to the factor (3). It is followed by the oxygen inactivation cross section, which steadily increases mainly due to the factor (4) for the neutron energies from 200 keV to $10^3$ keV.

It is noted that this good fit is mainly reflected from the fraction of hydrogen and oxygen atoms in water ($k_H = 0.667$ and $k_O = 0.333$) in the eq.(14).

The estimated inactivation cross section for the AT cells irradiated by fission neutrons is 92.2 $\mu$m$^2$, which is larger than the experimental value 78.6 $\mu$m$^2$. This over-estimation can be attributed to an overestimation of the yields of OH radicals as shown in eq.(2). The present model assumes the yields of OH for $^4$He$^{2+}$ ions as a function of $\lambda$ for all heavy charged particles. This assumption can be an origin of the overestimation of $\sigma(W-W)$, $\sigma(D-W)$ and $\sigma(W)$. For example, experimental $G(OH)$ is about 0.8 (molecules/100eV) at 10 ns for $^2$H$^+$(ions of energy 1 MeV/amu ($\lambda = 10.71$ nm), and 1.1 (molecules/100 eV) at 10 ns for $^3$He$^{2+}$ions of 4 MeV/amu ($\lambda = 10.66$ nm), while estimated G(OH) for protons of 1 MeV induced by fission neutrons ($E_n \sim 2$ MeV) is about 2 (molecules/100 eV) at 1 ns for the same $\lambda$ and this value is assumed in the present model. This kind of overestimation may appear especially for protons with an energy lower than 1 MeV ($\lambda < 10.71$ nm). More data on G(OH) for protons in this energy region is needed.

For aerobic conditions of structure C (Fig. 7) similar but slightly poorer agreements than structure B (Fig. 6) are seen. For components from the charged particles carbon and nitrogen nuclei arise additionally to those of hydrogen and oxygen nuclei. The number of hydrogen atoms is critical for the optimization of the model to the experimental data, especially for neutron energies $E_n < 1000$ keV and is a compromise over-estimation of OH yield for $^2$H$^+$. More data on $G(OH)$ for protons in this energy region is needed.
between two things, that the protein contains a relatively small fraction of the hydrogen and that some definite amount of hydrogen nuclei in the medium is needed for the component of the inactivation cross section as seen in Fig. 6. Our initial assumption of the equality of the number of atoms between water and protein inside the molecular target does not work, because the resulting fraction of hydrogen nuclei, 0.347, is not enough to explain the inactivation cross section for the neutron energies $E_n < 1000$ keV. The present result (Fig. 7) is given by an assumed fraction of the number of atoms such that the water:protein ratio equals 0.9:0.1 results in the fraction of hydrogen (0.603), carbon (0.0645), nitrogen (0.0157) and oxygen (0.3168), respectively. From this it is suggested that the DNA would be surrounded by a fairly large amount of water even in structure C, which is almost the same as that of structure B. This must be a future subject for checking with X rays crystallography.\(^\text{27}\)

From Figs. 8 and 9 for hypoxic conditions a similar insight to the aerobic conditions can be deduced except for the energy dependence in the region of $E_n < 1000$ keV. The contribution of the recoiled hydrogen nucleus is less obvious than that of the aerobic conditions (Figs. 6 and 7) because of the lack of experimental data. The ratio of the relevant number of atoms between water and protein is set as the same as for the aerobic conditions in structure C (Fig. 7).

The efficiency 6.8–7.4 of atomic deletion from DNA in hypoxic conditions is higher than the 3.1–4.6 for the aerobic conditions, which may imply that for aerobic conditions damages effected by charged particles are more irreparable due to oxygen fixation reactions and thus the relative importance of the damages induced by atomic deletion looks small, while for hypoxic conditions damages produced by charged particles have more of a chance to be repaired before operation of the oxygen fixation reactions and thus the relative importance of atomic deletion looks enhanced. It is, therefore, not known at the present whether the efficiency of the atomic deletion itself depends on the concentration of the dissolved oxygen.

As far as cell lethality by neutron irradiation is concerned, the inactivation cross sections for structure B may be a reasonable and important estimate (Fig. 10), and thus the biological, experimental data from hydrogen- and oxygen- ion irradiation in the relevant energy region are a basis for the study of mechanisms of biological effects induced by neutrons.

In summary cellular inactivation induced by fast neutrons consists of two components, one of which is the well discussed effects of secondary charged particles and the other of which is the newly suggested effects of atomic deletion that occurs in DNA (Fig. 10). It is estimated that the contribution

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Fig. 7. Inactivation cross section under aerobic conditions as a function of neutron energy estimated in terms of structure C. The estimated inactivation cross section $\sigma_{\text{total}}(E) = \eta \sigma_{\text{deletion}}(E) + \sigma_{\text{charged}}(E)$ is for repair proficient cell ($\eta = 4.8$). Experimental data are the same as Fig. 6. The components of $\sigma_{\text{charged}}(E)$ consists of those of hydrogen and oxygen from water and those of hydrogen, carbon, nitrogen and oxygen from the protein. The curves are grouped by atoms. The curves are the results when the number of atoms is set at a water : protein ratio = 0.9 : 0.1.
of the effect of atomic deletion can be as much as 15% (aerobic) or 55% (hypoxic) of the total cellular inactivation by fission neutrons (Fig. 11). It is also suggested that the severity of one event of atomic deletion is as much as 3.1–4.8 times (aerobic) or 6.8–7.4 times (hypoxic) the severity of one event induced by a single track of a secondary charged particle for irradiation by neutrons (\(\eta = 6.8\)). The legends for curves are the same as Fig. 6.

**Fig. 8.** Inactivation cross section under hypoxic conditions as a function of neutron energy estimated in terms of structure B. Experimental data on Tl\(^{51}\), P-388\(^{24}\) with neutron-energies reported are used to optimize the present model. The error bars are the maximum (14.3%) among aerobic data because no error is stated in references. The estimated inactivation cross section (solid curve) \(\sigma_{\text{total}}(E) = \eta \sigma_{\text{deletion}}(E) + \sigma_{\text{charged}}(E)\) is for repair proficient cells irradiated by neutrons (\(\eta = 6.8\)). The legends for curves are the same as Fig. 6.

**Fig. 9.** Inactivation cross section under hypoxic conditions as a function of neutron energy estimated in terms of structure C. The estimated inactivation cross section \(\sigma_{\text{total}}(E) = \eta \sigma_{\text{deletion}}(E) + \sigma_{\text{charged}}(E)\) is for repair proficient cells (\(\eta = 7.4\)). The setting of the ratio of water: protein atoms and the legends for curves are the same as Fig. 7.

**Fig. 10.** Inactivation cross section under aerobic conditions in terms of the same inactivation cross section in Fig. 6 is shown including results for lower neutron energies. The legends for curves are the same as Fig. 6.
or by heavy ions (especially H and O) for the relevant energy regions would be very useful to study this relative severity more quantitatively.

**Cell transformation induced by neutrons**

To investigate the relationship between cellular inactivation and other biological endpoints, data from cell transformation studies22 are analyzed by the present model. A similar good reproduction of the data with the present model is seen in Fig. 12. The related optimized parameters are given in Table 1 and also the saturated cross sections, which are $\sigma = (96.1\pm 2.2) \times 10^{-4} \mu m^2$ (structure B), $\sigma = (98.0\pm 7.4) \times 10^{-4} \mu m^2$ (structure C), respectively. In Fig. 12 the same number of atoms in water and protein inside the molecular target as for the case of inactivation (Fig. 7) is used. The significance of atomic deletion is slightly higher than that for inactivation (structure C) as shown in Table 1. This may contain a message on the biological consequences of atomic deletion.

**RBE of cellular effects by neutrons**

Eq.(17) calculates neutron LET in terms of the primary LET of the secondary charged particles set in motion by elastic scattering. LET for relevant biological materials are calculated by a similar way as eq.(17) and are shown in Fig. 13, as protein (dotted curve), structure C (dashed curve), chromatin (long dashed curve), ICRU tissue24 (solid curve) and water (a half dash curve, structure B) respectively. The order of LET is roughly proportional to the amount of carbon atoms in the substance. Dosimetry with ICRU tissue or water will tend to underestimate the dose to chromatin, though these kinds of discussion using the concept of LET
are questioned. RBE can be calculated by the ratio of radiosensitivity of neutrons to that of the reference radiation. Radiosensitivity can be estimated by relevant cross sections using \( \sigma_{\text{estimation}} \) and calculation of the absorbed dose in water. Radiosensitivity of the reference radiation, which is used in the present work, is the experimental value for 250 KVP X-rays for repair proficient cells.

RBE are as shown in Figs. 14 and 15. The results are comparable with experimental estimates and deepen the discussions of neutron RBE by showing its components in terms of recoiled charged particles. Biological experiments with neutrons of energies 1 keV < \( E_n < 200 \) keV are important to check the present estimates of RBE. It is also noted that the estimated RBE for protons at energies around \( E_p = 50 \) keV are the highest among charged particles when they are starters inside the cell nucleus.

The present estimation of RBE for the cell transformation is compared with the radiation weighting factor for neutrons (Fig. 16). For neutrons of energies \( E_n < 100 \text{keV} \) and \( E_n > 2 \text{MeV} \) the radiation weighting factor may correspond to the RBE of the recoiled charged particles, while for neutrons of \( 100 \text{keV} < E_n < 2 \text{MeV} \) the radiation weighting factor may cover the estimated total RBE, which includes the component of the atomic deletion. More study on the radiation weighting factor for the case of \( E_n < 100 \text{keV} \) and \( E_n > 2 \text{MeV} \) is clearly needed.

As mentioned above, quantitative relations between two radiations of different qualities should be interpreted carefully, for instance, the ratio of relevant quantities (absorbed doses for the same level of effect or radiosensitivities) may be valid if the underlying mechanisms are at least based on common ground (electronic interactions). The possible existence of an atomic deletion mechanism in neutron exposure indicates an inherent deviation from this common ground, and it is strongly recommended to study the biolog-
ical impact specific to this effect and to find a quantitative relation to the concept of absorbed dose for this effect.

DISCUSSIONS

Overview of neutron cellular inactivation from the present model

In Fig. 10 cellular inactivation under aerobic conditions is shown including intermediate energy neutrons, where neutrons are classified by energy as thermal neutrons \( \text{En} < 0.5 \text{ eV} \), intermediate neutrons \( 0.5 \text{ eV} < \text{En} < 10 \text{ keV} \) and fast neutrons \( \text{En} > 10 \text{ keV} \). In the region of intermediate neutrons the inactivation increases steadily as neutron energy increases with atomic deletion as the dominant effect. In the region of fast neutrons it is seen that the cellular inactivation by secondary charged particles dominates steadily in the total response as neutron energy increases and that a clear shoulder is observed in the energy range 20 keV–4 MeV. Because of the existence of a local minimum of the inactivation due to secondary charged particles induced by fission neutrons (\( \text{En} \sim 2 \text{ MeV} \)), a relatively higher contribution from atomic deletion is seen (Fig. 11). In the intermediate neutron energy range inactivation decreases dramatically as neutron energy decreases. Estimated inactivation cross section for repair proficient cells is about \( 0.01 \mu m^2 \) at neutron energies of \( \text{En} \sim 0.03 \text{ keV} \) that is comparable to that of \( ^{137}\text{Cs} \) or \( ^{60}\text{Co} \) gamma rays (Fig. 7(a) in the ref.14). The present model, however, draws attention to the fact that the underlying mechanisms are totally different for each radiation type even if their inactivation cross sections are of the magnitude. For thermal neutrons the capture reactions \( ^1\text{H}(n,\gamma)^2\text{D} \) and \( ^{14}\text{N}(n,p)^{14}\text{C} \) become significant at constituent atoms of DNA and their consequent “atomic mutation” and the secondary gamma rays and proton may cause DNA radiation damages. This will be an important future subject of study.

Components of the inactivation cross section induced by atomic deletion

It is interesting to see whether some specific structural residue of DNA among base, sugar and backbone dominates or not the inactivation cross section for atomic deletion. Inactivation cross section for each residue is calculated (Fig. 17). For neutron energies lower than 400 keV the magnitude of the inactivation cross sections for these residues of DNA are base, sugar and backbone in that order, while for neutron energies higher than 400 keV the magnitudes, in order, are base, backbone and sugar (Fig. 17). Further the components of the inactivation cross section for each type of base. G, A, T and C results in this order of magnitude. It is concluded that no residue of DNA exists specific to the total effect of the atomic deletion. The inactivation cross section of the atomic deletion for each type of atomic constituents of DNA is given (Fig. 18) addressing the question whether some specific type of atom of DNA is most responsible or not for the effect of atomic deletion. For a neutron energy of \( \text{En} < 200 \text{ keV} \) the atomic deletion occurs dominantly at hydrogen atom, while for neutron energy higher than this energy the atomic deletions occurs at C, O, N, and P atoms, depending on the neutron interaction cross section and number of atoms in DNA. The question whether efficiencies to the biological endpoint from each type of atomic deletion are the same or not is also an important subject for future study.
Further improvement of the present model

The proposed model should be improved in a number of ways. (1) Application of the neutron energy spectrum instead of a nominal energy of the neutron. (2) The energy spectrum of the secondary particles taken into consideration instead of their average value. (3) Consideration of particles that emerge from inelastic scattering. (4) A molecular model of structure C for DNA is built from data from X-ray crystallography instead of the current simpler model, including realistic atomic composition data. (5) Finally, estimation of cellular effects (cell inactivation, cell transformation, mutation etc.) for repair proficient cells by neutrons depends on estimations for heavy charged particles, confidence in which may depend on modeling how well repair processes can be quantitatively explained.

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