Kinetic model of random DNA cleavage by radiation

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A kinetic model of random DNA cleavage induced by radiation is presented. The method is distinct from the commonly used combinatoric technique orginated by Montroll and Simha some time ago. To demonstrate its flexibility, application is made to fragmentation of ring molecules. Having an alternative way of describing random scission processes should be of some benefit in formulating more detailed models. [S1063-651X(97)04011-7]

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I. INTRODUCTION

Advances in the ability to observe directly the radiation-induced DNA double-strand breakages have generated renewed interest in describing the mechanisms for such breakages and their repair. With atomic-force microscopes, it is possible to see fragmented DNA strands and measure their lengths with resolutions on the order of a nanometer [1]. This ability gives us a new window to see how direct and indirect radiation damage is inflicted onto genes.

An important observable that a radiation-damage theory can predict is the distribution of fragment lengths created by a given exposure and given environment. There is an extensive literature in which statistical methods are used to describe DNA fragmentation by radiation both *in vitro* and *in vivo*, including an account of microdosimetry with given molecular geometries [2]. These works focus on understanding the details of radiation-induced damage using mechanistic descriptions of radiation-track formation and subsequent effects in biologically active molecular system.

This paper presents an alternative starting point for the description of fragmentation processes based on their time evolution. The intention is not to replace more detailed models, but rather to suggest a different way to think about the physics of the processes. Some examples are more easily formulated in terms of this ''kinetic'' model. For the case of an initial distribution of fixed length chains of monomers (nucleotides), the model agrees with the results of Montroll and Simha [3] who used a completely different method based on statistical combinatorics. As an example of the flexibility of the present kinetic formulation, the fragmentation distribution for an initial set of ring plasmids is also derived.

II. CLEAVAGE OF LINEAR MOLECULES

Consider an initial volume of DNA molecules dispersed in a sample volume of target material, such as a water solution. If this volume is irradiated, causing locally deposited energy sufficient to break both backbones of the double helix (directly or indirectly), then cleavage will have some probability of occurring. (Here, direct break up refers to cleavage caused by energy deposited onto the DNA from primary and secondary radiation while indirect break up includes energy transferred from nearby ions and radicals created by the radiation.) To model the radiation-induced cleavage events, assume that the molecule is made of segments of minimum length δ, and that the initial molecules have length $n\delta$, where n is an integer. Let N_i be the number of DNA molecule fragments present at a particular time having a length $i\delta$. Initially, all N_i are zero except N_n, which starts as the total number of DNA molecules present in the initial volume, which is taken as N. As the molecules are irradiated, the change in the number of molecules having length $i\delta$ comes about from two mechanisms: an increase from cleavage of larger molecules and a decrease due to cleavage of the molecules of length $i\delta$. For uniform radiation of randomly distributed molecules all equally exposed to the radiation, the rate of cleavage of the molecules of length $i\delta$ will be proportional to the number of possible cleavage points, $i-1$. (For simplicity, fixed length monomers and equal probability for site breakage is taken. These assumptions can be relaxed without reformulating the method.) A single cleavage of the molecules of length longer than $i\delta$, say $k\delta$, will have an equal chance to make fragments of length from δ to $(k-1)\delta$. From the set of such single-cleavage possibilities of a given molecule, two of this set will produce segments of length $i\delta$, adding to the number with this length.

It follows that

$$\frac{dN_i}{dt} = -(i-1)rN_i + 2r\sum_{k=i+1}^{n} N_k, \tag{2.1}$$

where r is the rate at which the given radiation causes a cleavage at a given site of the DNA molecule. In particular,

$$\frac{dN_n}{rdt} = -(n-1)N_n,$$

$$\frac{dN_{n-1}}{rdt} = -(n-2)N_{n-1} + 2N_n,$$

$$\frac{dN_{n-2}}{rdt} = -(n-3)N_{n-2} + 2(N_{n-1}+N_n),$$

$$\cdots$$

$$\frac{dN_1}{rdt} = 2(N_2+N_3+\cdots+N_n). \tag{2.2}$$

The solutions for the N_i that satisfy the initial conditions (at $t=0$)

$$N_n(0)=N, \tag{2.3}$$

$$N_i(0)=0 \quad \text{for } i<n$$

can be found in a straightforward manner, starting with N_n, and leading to

$$N_n=Ne^{-(n-1)rt},$$

$$N_{n-1}=N(-2\,e^{-(n-1)rt}+2\,e^{-(n-2)rt}),$$

$$N_{n-2}=N(1\;e^{-(n-1)rt}-4\,e^{-(n-2)rt}+3\,e^{-(n-3)rt}),$$

$$N_{n-3}=N(2\,e^{-(n-2)rt}-\;6\,e^{-(n-3)rt}+4\,e^{-(n-4)rt}),$$

$$\cdots$$

$$N_2=N[(n-1)e^{-rt}-2\,(n-2)e^{-2\,rt}+(n-3)e^{-3\,rt}],$$

$$N_1=N[2(n-1)(1-e^{-rt})-(n-2)(1-e^{-\,2\,rt})] \tag{2.4}$$

or, in general,

$$N_l=N[\overline{\delta_{nl}}(n-l-1)e^{-(l+1)rt}-2(n-l)e^{-lrt} +(n-l+1)e^{-(l-1)rt}], \tag{2.5}$$

where $\overline{\delta_{nl}}$ excludes $n=l$, i.e., $\overline{\delta_{nl}}=(1-\delta_{nl})$, and δ_{nl} is the Kronecker delta. Note that as t approaches ∞,

$$N_l\to 0 \quad (l>1), \quad N_1(t)\to nN, \tag{2.6}$$

so that after sufficient time, all of the original N molecules have been divided into n segments of length δ. These solutions also satisfy

$$\sum_{l=1}^{n} lN_l(t)=nN \tag{2.7}$$

for all time t, showing that the total length of all the broken segments remains unchanged. Atomic-force microscopy can be used to determine N_l by direct measurement. Techniques that separate fragments according to their molecular weight, such as electrophoresis, determine the distribution $f_l\equiv lN_l$.

From the form of the predicted number of original lengths (having $n-1$ cleavage sites),

$$N_n=N(e^{-rt})^{n-1},$$

the expression e^{-rt} may be interpreted as the probability that a given site is not cleaved by the radiation. The probability a given site becomes cleaved is then

$$\alpha\equiv 1-e^{-rt}, \tag{2.8}$$

so that from Eq. (2.5) the number of segments present with length l (for $l<n$) is

$$N_l=N\alpha(1-\alpha)^{l-1}[2+(n-1-l)\alpha] \tag{2.9}$$

while for $l=n$,

$$N_n=N(1-\alpha)^{n-1}. \tag{2.10}$$

Equations (2.9) and (2.10) agree precisely with the results of Montroll and Simha [3]. (See also the related works of Sakurada and Okamura [4] and of Charlesby [5].) However, the above derivation is far simpler than the combinatorical methods used by Montroll and Simha. Their parameter α, the average fraction of cuts in each original molecule, i.e., the frequency of cleavage, can be given the physical interpretation as the probability of molecular cleavage at a given site. In turn, the probability that a given site remains uncleaved is the inverse exponential of the ''cleavage number,'' rt, which is the product of the rate of cleavage of a given site and the exposure time. The Appendix makes a connection between the radiation dose and the cleavage number rt.

III. CLEAVAGE OF PLASMIDS

Suppose the DNA starts as a ring plasmid of fixed circumference $n\delta$, which can be broken by the radiation at n vulnerable sites. Then the supply of two-ended lengths after irradiation comes from double-strand breakage of these plasmids. Taking the rate of plasmid breakage proportional to the number of possible breakage sites n, the number of still-unbroken plasmids at time t will be

$$N_{\text{ring}}=Ne^{-nrt}, \tag{3.1}$$

where r is again the rate of double-strand breakage due to radiation when only a single site near or on the DNA is exposed. Let N_l be the number of fragments of length $l\delta$ produced by the breakup of the plasmids. This number changes by loss through the $(l-1)$ ways for further cleavage and from breakup of larger fragments, there being two ways to produce length $l\delta$ from lengths $k\delta$ $(n\geqslant k>l)$. Thus

$$\frac{dN_l}{rdt}=-(l-1)N_l+2(N_{l+1}+N_{l+2}+\cdots+N_n) \tag{3.2}$$

for $l<n$, while

$$\frac{dN_n}{rdt}=-(n-1)N_n+nN_{\text{ring}}. \tag{3.3}$$

These have the explicit solution

$$N_l=nN(e^{-(l+1)rt}-2\,e^{-lrt}+e^{-(l-1)rt}) \tag{3.4}$$

for $l<n$ and

$$N_n=nN(e^{-(n-1)rt}-e^{-nrt}). \tag{3.5}$$

As expected, the N_l satisfy conservation of segment length at all times given by

$$\sum_{l=1}^{n} lN_l+nN_{\text{ring}}=nN. \tag{3.6}$$

In terms of the average fraction α of cuts per molecule [the same α introduced in Eq. (2.8)],

$$N_l=nN\alpha^2(1-\alpha)^{l-1}, \tag{3.7}$$

$$N_n = nN\alpha(1-\alpha)^{n-1}, \tag{3.8}$$

and

$$N_{\text{ring}} = N(1-\alpha)^n. \tag{3.9}$$

The slope of the fragment population as a function of length becomes

$$\frac{\Delta N_l}{\Delta l} = -4nNrte^{-lrt}\sinh^2\frac{rt}{2}, \tag{3.10}$$

which is always negative and diminishes toward zero for the longer-length fragments.

The number of split but unfragmented molecules initially grows linearly with the cleavage rate,

$$N_n = nNrt - \tfrac{1}{2}nNr^2t^2(2n-1) + \tfrac{1}{6}nNr^3t^3(3n^2-3n+1)\cdots. \tag{3.11}$$

Interestingly, for low doses, the population of fragments does not have the first-power dose dependence of the linear-molecule case, but rather starts with a quadratic behavior in rt:

$$N_l \simeq nNr^2t^2(1-lrt+\cdots) \tag{3.12}$$

for $l<n$ and $lrt \ll 1$. In the case for which two localized radiation events are required for double-strand breakage, rt will be proportional to the square of the dose (see the Appendix), so N_l will have a quartic initial dependence on dose.

IV. CONCLUSIONS

A kinetic model of DNA cleavage induced by radiation is a useful alternative to the standard combinatoric model. When applied to linear molecules, it agrees with the combinatoric-statistical theory of Montroll and Simha. The kinetic-model method is quite flexible, allowing the inclusion of different rates of cleavage along a given molecule and a variety of initial states. For example, the fragmentation population produced by irradiating DNA plasmids can be described. Direct measurement of fragmentation lengths, now possible with atomic-force microscopy, lets us see directly how the fragment population depends on dose, and therefore we can unambiguously answer under what circumstances double-strand breakage of DNA by radiation is dominated by single-hit events and when double events come to play.

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APPENDIX: FRAGMENTATION AND DOSE

In this appendix, a connection is drawn between the radiation dose given to a sample of DNA and the cleavage probability. This will allow us to express the fragmentation numbers in terms of the radiation suffered by the sample. At this stage, a simple but reasonable target model will be taken in order to make the relationships clear.

Consider dividing the sample into M volumes (''sites''), each of size v, small enough so that, if radiation of sufficient energy is deposited into one site surrounding a location on a DNA molecule, it will cause a cleavage of the DNA. Suppose the radiation generates m localized deposition events randomly distributed throughout the sample and of sufficient energy to cause a cleavage. The probability that a given site is hit exactly k times by the first k events will be $(1/M)^k$. The chance that the remaining $(m-k)$ radiation events hit the other sites is $(1-1/M)^{m-k}$. But the k events on a given site may have occurred interspersed in time among the m depositions events. There are $(m-k+1)(m-k+2)\cdots m$ ways in which an ordered set of the k events in the given site could have occurred among the remaining $(m-k)$ events that did not hit the given site. Any ordering of the k events is equivalent, so that the number of ways that an unordered set of the k events can occur is $(m-k+1)(m-k+2)\cdots m/k!$. Therefore, the probability that a given site experiences exactly k hits after m events among M loci is

$$p_k = \binom{m}{k}\left(\frac{1}{M}\right)^k\left(1-\frac{1}{M}\right)^{m-k}, \tag{A1}$$

where $\binom{m}{k}$ is the binomial coefficient. The probability that a given locus is hit one or more times will be

$$\sum_{k=1}^{m} p_k = 1-\left(1-\frac{1}{M}\right)^m \tag{A2}$$

and the probability that it is hit two or more times will be

$$\sum_{k=2}^{m} p_k = 1-\left(1-\frac{1}{M}\right)^{m-1}\left(1+\frac{(m-1)}{M}\right). \tag{A3}$$

If only one hit in a localized volume is required to cause (directly or indirectly) a cleavage of DNA, then the Montroll-Simha parameter α, i.e., the probability that a given site is broken, becomes

$$\alpha_1 = 1-\left(1-\frac{1}{M}\right)^m. \tag{A4}$$

If two or more hits are required, then α becomes

$$\alpha_2 = 1-\left(1-\frac{1}{M}\right)^{m-1}\left(1+\frac{(m-1)}{M}\right). \tag{A5}$$

In terms of the cleavage number, rt, Eq. (2.8) gives

$$r_1 t = m\ln\left(\frac{1}{1-1/M}\right) \approx \frac{m}{M} \tag{A6}$$

and

$$r_2 t = (m-1)\ln\left(\frac{1}{1-1/M}\right) - \ln\left(1+\frac{m-1}{M}\right)$$
$$\approx \frac{m}{M} - \ln\left(1+\frac{m}{M}\right) \approx \frac{m^2}{2M^2}, \tag{A7}$$

where the last approximations follow by taking $M \gg 1$ and $M \gg m \gg 1$.

Now the dose D of radiation left in the sample is the total energy deposited per unit mass of sample. The number of localized energy deposits left in the sample, m, should be proportional to the dose over a wide range of exposures as

$$m = \frac{\mathcal{M}}{\varepsilon} D, \tag{A8}$$

where $\mathcal{M}$ is the mass of the sample and ε is defined by this relation and measures the energy needed for a double-strand cleavage. From this connection between dose and number of localized energy deposits, the rate of site cleavage will be proportional to the dose if only a single hit is needed. In contrast, if two hits within a given site are needed, then the rate of site cleavage will be proportional to the square of the dose. If the sample has mass density ρ, then

$$M = \frac{\mathcal{M}}{\rho v}, \quad \text{making} \quad \frac{m}{M} = \frac{\rho v D}{\varepsilon}. \tag{A9}$$

It then follows from Eq. (A6) that

$$r_1 t = \frac{\rho v D}{\varepsilon_1}, \tag{A10}$$

while, if $M \gg m$, Eq. (A7) gives

$$r_2 t = \frac{1}{2}\left(\frac{\rho v D}{\varepsilon_2}\right)^2. \tag{A11}$$

Since, under the second scenario, two or more energy deposits are needed for cleavage, the average ε_2 should be about half of ε_1. The expressions for rt, given in Eq. (A6) and Eq. (A7), determine the dependence of the DNA fragmentation numbers, N_l [Eqs. (2.9)–(2.10), (3.7)–(3.9)], on radiation dose. In terms of the frequency of cleavage α (even if $\rho v D/\varepsilon$ is not small),

$$\alpha_1 = 1 - e^{-\rho v D/\varepsilon_1} \tag{A12}$$

and

$$\alpha_2 = 1 - e^{-\rho v D/\varepsilon_2}(1 + \rho v D/\varepsilon_2). \tag{A13}$$

If $\rho v D/\varepsilon \ll 1$ (low doses), then $\alpha_1 \sim \rho v D/\varepsilon_1$ and $\alpha_2 \sim (\rho v D/\varepsilon_2)^2/2$. The ratio α/D will be constant with dose for a given sample only for small doses and only in the case of single-event cleavages.

To appreciate the size of these numbers, let us estimate the magnitude of the expression in Eq. (A10). Suppose DNA molecules are in a water solution and are given a dose of 100 Gy. The density ρ is approximately that of water, 10^3 kg/m^3. Note that the volume v is not the size of the primary and possible secondary ionization volumes covering an ionization track, but rather the volume surrounding a DNA site, which, if sufficient energy is deposited within, causes cleavage. If we take the radius of the interaction volume to be 5 nm, then v will be approximately 10^{-25} m^3. Now take the effective energy ε needed in the volume v to cause a double-strand break to be 25 eV. (The threshold energy has been measured to be about 8 eV for photons, with 20 to 30 eV needed for electrons [6].) Then $r_1 t \sim 0.01$. With the cleavage number rt much less than 1, rt will be close to the average fraction of cuts α. Even so, the number of possible cleavage sites n along a DNA molecule can be much larger than 10^2, so that the exponents in Eqs. (2.5) and (3.4) must be used for the large fragments ($l \sim n$), rather than their small rt approximation. Measurements for gamma-ray irradiation of mammalian DNA give rather small values for α. Friedl [7] reports α/D in the range $(6 \pm 2) \times 10^{-9}$ double-strand breaks per Gray per base-pair. With an effective energy deposit of 8 eV, the radius of the interaction volume if breakage had been dominated by a single-hit would be under 0.2 nm while a double-hit breakage at 100 Gy would give an interaction radius near 2 nm.

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