A mathematical model of DNA fragmentation induced by gamma-rays

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Abstract: - A suitable modelling of the radiation-induced DNA fragmentation may greatly help in developing a clearer biophysical description of radiation action in mammalian cells. We have developed a model aimed at describing the DNA fragment distribution induced by ionising radiation. It is based on a random fragmentation formalism already developed in the literature, generalized in such a way to be applied to any initial size distribution. This model has been applied to the DNA breakage induced in mammalian V79 cells by 25 Gy γ-rays, as determined by the pulsed-field gel electrophoresis technique in the size range 23 kbp – 5.7 Mbp. The usual random breakage model, based on the assumption of initial monodisperse population of molecules, strongly disagrees with the experimental results, while our model gives a quite good description of the observed pattern when the experimental distribution in the non-irradiated sample is taken as the initial distribution in the model.

Key-Words: - DNA, radiation-induced damage, fragmentation, double-strand breaks, pulsed field gel electrophoresis, ionising radiation, γ-rays, V79 cells

1 Introduction

A great deal of work has been devoted to characterization of damage induced by radiation into cellular DNA and its quantification, paying a special attention to double strand breaks (DSB) of the helix, since they are considered as the most biologically relevant lesions in yeast and mammalian cells [1,2].

In recent years much interest has been addressed to the correlated damage in cellular DNA induced by ionising radiation, in order to get a deeper insight of the basic mechanisms of radiation action. The degree of spatial correlation may have important biological consequences, since it is expected that it affects the cell ability to repair DNA lesions.

An important issue is whether or not this damage is distributed at random along the DNA, taking into account the interplay between radiation track structure and chromatin organization. Several characteristic distances for damage correlation can be considered, related to various levels of chromatin organization. The DNA in mammalian cells is organized in nucleosomes, linked together to give a filament folded into a chromatin fiber that in turn is organized in loops associated to the nuclear matrix.

Among the various methods so far developed to detect the extent of DNA breakage, gel electrophoresis in the presence of a constant (constant field gel electrophoresis, CFGE) or pulsed (pulsed field gel electrophoresis, PFGE) electric field is now widely used. The latter method is capable to analyze relatively large DNA fragments that cannot be separated by the first one. Some experiments based on such methods have shown that the random breakage models in their usual forms are inadequate to describe the distribution of DNA fragments in irradiated mammalian cells [3,4]. This discrepancy is larger with densely ionising radiation (helium and heavier ions) than with sparsely radiation (X- or γ-rays).

However, since also the control (non-irradiated) samples are not monodisperse, showing non-random distribution, the question can be raised whether the observed non-random distributions of fragments in the irradiated samples are due to the presence of non-random fragmentation in the initial (non-irradiated) samples. A suitable modelling of the radiation-induced DNA fragmentation may greatly help in developing a more accurate biophysical description of radiation action in mammalian cells.

In this paper we describe a model of DNA fragmentation based on a modification of the random fragmentation model in order to take into account the initial distribution of the DNA molecules. We apply the model to describe the fragmentation profile obtained by PFGE on mammalian V79 cells exposed to γ-rays.

2 Matherials and Methods

We give some details on the experimental procedures in the first subsection; then, in the following two subsections, we introduce the model, and in the last subsection we show the results obtained by applying the model to our experimental data.

2.1 Experimental procedures

Chinese hamster V79 cells, whose DNA had been labelled with ^{14}C, were irradiated in monolayer using a ^{60}Co γ rays source at a dose rate of about 15 Gy/min. During irradiation cells were kept at 4°C. Cells were then detached by trypsin-EDTA treatment performed on ice, centrifuged, resuspended in low-gelling agarose and lysed in the presence of proteinase K and detergents.

Calibrated PFGE was used to analyse the DNA fragments in the size range 5.7 – 0.023 Mbp. PFGE was performed using a Bio-Rad CHEF-DRIII system under two different conditions able to optimise separation in the size ranges 5.7-1.0 Mbp and 1.0-0.023 Mbp, respectively. The results were combined together after proper normalization. Calibration was accomplished by using DNA from S. pombe, S. cerevisiae, λ ladder and λ-HinDIII as size markers. More details can be found in a forthcoming paper [5].

The fragmentation profiles were evaluated by dividing the gel in a number of zones and measuring the fraction of DNA mass (in terms of Fraction of Activity Released, FAR) entering the gel in each zone. A quantity proportional to the number of fragments in each zone was determined by dividing the fraction of DNA mass in that zone by the corresponding average mass. The frequency distribution of fragments per unit mass was then obtained as the ratio of the number of fragments to the size interval encompassed by each zone. The yield for induction of DNA DSB was evaluated by summing up the number of fragments induced in each zone.

2.2 The broken stick model

The starting point of our modelling is the broken stick model studied by several authors [6,7,8]. and later considered again and applied to gel electrophoresis in [9]. It is based on the following assumptions: 1) initially identical molecules; 2) randomly inserted breaks; 3) continuous fragment distribution (well approximated when fragment mass is still much higher than a base pair).

Suppose to irradiate a DNA segment of mass M_0 (here and in the following we will measure the length of a fragment by its mass, which is obviously proportional to the number of base pairs). The radiation will produce an average number of DSB per initial molecule that will be denoted by $\mathbf{m}$, which is proportional to the dose d and to the mass M_0 through the yield y, the number of DSB per unit mass and unit dose: $\mathbf{m}=y\cdot d\cdot M_0$. Then one obtains the following distribution:

$$F(M)dM = \left\{ \frac{\mathbf{m}}{M_0}\left[2+\mathbf{m}\left(1-\frac{M}{M_0}\right)\right]\exp\left(-\mathbf{m}\frac{M}{M_0}\right)+\right.$$
$$\left. +2\exp(-\mathbf{m})\mathbf{d}(M_0-M)\right\}\mathbf{c}(M_0)dM \qquad (1)$$

which is the number of fragments with mass between M and $M+dM$. In equation (1) $\mathbf{c}(M)$ is the characteristic function, which is 1 between 0 and M and 0 otherwise. In the first line of equation (1) we have the term that arises when the segment is really hit; in the second line there is the term that takes into account the probability that the segment is not hit, and the fragment produced is obviously the same M_0. The number of hits follows the Poisson distribution with average $\mathbf{m}$, and then the last probability is equal to $\exp(-\mathbf{m})$. The factor 2 in the term with the Dirac $\mathbf{d}$ function corrects for the fact that, due to the presence of $\mathbf{c}(M_0)$, the mass integral of $F(M)$ is actually extended between 0 and M_0, and then the integral of the $\mathbf{d}$ function gives 1/2. The normalization of $F(M)$ is such that the mass integral of equation (1) is equal to $1+\mathbf{m}$, which is the number of fragments produced by $\mathbf{m}$ DSB.

The above treatment applies when all the fragments produced by the radiation are given by the fragmentation of a monodisperse sample, i.e., all the initial segments have the same mass M_0. When the sample is polydisperse, and the masses of the initial segments follow a given distribution, the treatment has to be generalized.

2.3 The generalized broken stick (GBS) model

Suppose to have a distribution $g(M_0)$ for the mass of the initial segments exposed to the radiation. We do not make any hypothesis on the dstribution $g(M_0)$, in particular we do not suppose any randomness. For the following formal development $g(M_0)$ can be considered arbitrary; in the application to our PFGE experiments we will explain how it is obtained. The function $g(M_0)$ is normalized:

$$\int_0^\infty g(M_0)dM_0 = 1 \qquad (2)$$

The integral is formally extended between 0 and infinite. In real cases the masses will be distributed between two extreme values; however, this can be

included in the function $g(M_0)$, which will be different from 0 only between these extremes.
A fragment of mass M can of course be produced only by the fragmentation of a segment with mass at least M. To generalize the model we add M_0 as a parameter to the notation of the function $F(M)$ defined in (1), to take into account the mass of the initial segment; it becomes $F(M;M_0)$. We now indicate with $F_T(M)$ the number of fragments per unit mass obtained after irradiation of the initial segments. Following the above considerations we will have:

$$F_T(M) = \int_M^{\infty} g(M_0) F(M; M_0) dM_0 \qquad (3)$$

It can be easily verified that also the mass integral of $F_T(M)$ is equal to 1+**m**

2.4 Application of the GBS model to the experimental PFGE distributions

The measured FAR in the i-th zone of the gel is denoted with f_i. This number, as previously noted, is also equal to the fraction of mass in that zone. The mass values corresponding to the limits of the zones in the gel are determined by the use of markers. The difference of the masses corresponding to the two extremes of the i-th zone is denoted by **D**M_i, and the average mass by m_i. From these quantities we define the experimental distribution of fragments per unit mass as:

$$F_i^{\exp} = \frac{f_i}{m_i \Delta M_i} \qquad (4)$$

These experimental data have been obtained in the size ranges 5.7-0.023 Mbp, as noted before. The same measure has been performed without irradiation, to determine the distribution of the length of the initial segments $g(M_0)$. In order to apply expression (3) one has to know $g(M_0)$ also outside this range. Since we have found that, inside the experimental range the function is well fitted by a linear interpolation in double logarithmic scale, we have used the same linear fit to find, by extrapolation, the values of $g(M_0)$ outside the experimental range.

The quantity in (4) has to be compared to the theoretical value. We denote with S the maximum value of the mass of the initial segments (namely, the mass of the entire average chromosome). S is then the mass above which the distribution $g(M_0)$ is identically 0. The theoretical distribution is readily obtained from (3) through:

$$F_i^{theor} = \frac{F_T(m_i)}{S} \qquad (5)$$

We show here the comparison for the data obtained after irradiating the cells with a dose of 25 Gy of γ-rays. The results are shown in Figure 1. It can be seen that our GBS model gives a good description of the experimental results, when the experimental distribution in the non-irradiated control is taken as the initial distribution in the model.

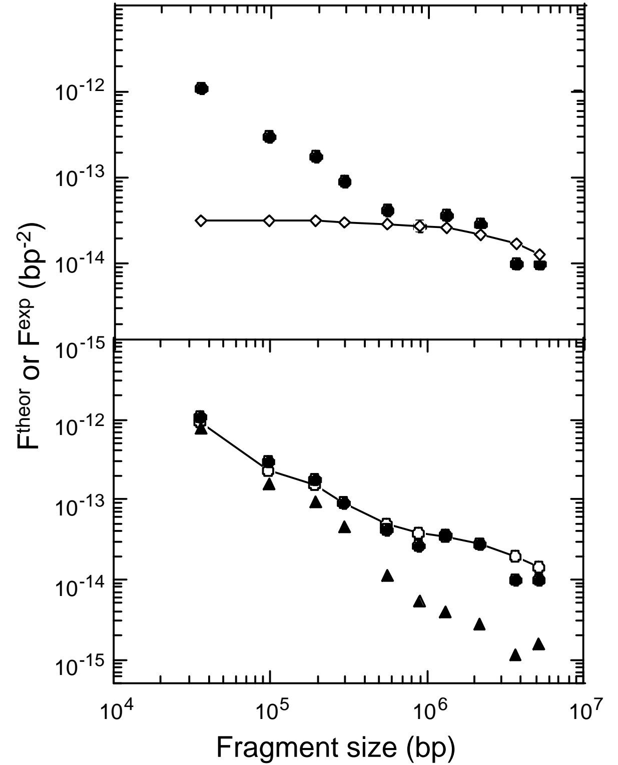

Figure 1. Upper panel.: experimental frequency of DNA fragments induced by 25 Gy of γ-rays (solid circles) compared to the prediction of the broken stick model for the monodisperse case (open diamonds connected by a line). Lower panel: experimental frequency as above, compared to the prediction of the GBS model (open circles connected by a line) using as the initial distribution that in the non-irradiated samples (solid triangles).

3 Conclusion

The experimental frequency distribution of DNA fragments observed in the size range 5.7 – 0.023 Mbp after cell irradiation has a monotone shape without peaks that are instead predicted and observed at sizes smaller than few kbp for sparsely ionising radiation [10,11,12]. These short fragments reflect the chromatin organization at the nucleosome and fiber levels that are not relevant for the size range considered in this study. Nevertheless, our experimental distribution cannot be described by a random breakage mechanism acting on an initially monodisperse distribution.
Two main sources of non-randomness can be envisaged: i) non-random distribution of the

radiation-induced breaks, and/or ii) presence of non-random fragmentation in the initial (non-irradiated) sample. Occurrence of ii) is clearly shown by the fragment distribution of the control (non-irradiated) sample.

Simulation of fragmentation by our generalized random breakage model gives quite a good agreement with the experiments when the control distribution is taken as the initial distribution of the model.

In conclusion, description of DNA breakage induced by sparsely ionising radiation does not require any non-random mechanism, provided that the initial DNA distribution is taken into account.

This conclusion may not hold for densely ionising radiation, such as protons, helium ions and heavier ions, since it is expected that there is an interplay between their track structure and the chromatin organization at the loop level, with a consequent influence on the production of fragments in the 10-10^3 kbp region [4,5].

Work is in progress to better understand the origin of the fragmentation observed in the non-irradiated samples and to simulate in a more realistic way the initial DNA distribution by considering the actual chromosome sizes in V79 cells.

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