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Abstract. Quantification of DNA damage, induced by various types of incident radiation as well as chemical agents, has been the subject of many theoretical and experimental studies, supporting the development of modern cancer therapy. The primary observations showed that many factors can lead to damage of DNA molecules. It became clear that the development of experimental techniques for exploring this phenomenon is required. Another problem was simultaneously dealt with, anticipating on how the damage is distributed within the double helix of the DNA molecule and how the single strand break formation and accumulation can influence the lethal double strand break formation. In this work the most important probabilistic models for DNA strand breakage and damage propagation are summarized and compared.

1. Introduction

Although for many years DNA damage has been subject to various studies, in recent years a new approach has been undertaken in order to assess the influence of radiation and chemical agents action on DNA. Breakage of the DNA double helix has been investigated under various conditions when exposed to both ionizing and non-ionizing radiation as well as other reactive species, like hydroxyl radicals or cutting and nicking enzymes. In all these attempts the main goal was to be able to obtain highest levels of double-strand breaks (DSBs), lethal to living cells. It was also found that accumulation of single strand breaks (SSBs) can lead to DSBs appearance and further to cell death, when the single-strand lesions appear in both strands within a small distance, h. This distance is measured in basepairs for double stranded DNA molecule. It describes a span between SSBs created in opposite strands that will lead to hydrogen bond rupture between complementary bases lying between these breaks and further create a DSB in DNA molecule. Together with changes in DNA conformation, this distance became one of the most important parameters to be determined in samples exposed to damaging agents of various kinds.

Simultaneously, investigations on the probability of breaking a DNA strand by incident particles were conducted. In early years the damage distribution assessment was based on probabilistic models. The very first studies accounted for neither parameters like random lesion distribution within both strands nor the strength of phosphodiester bonds in the DNA backbone [1], and the experimental data fitted to the theoretical model that was developed gave a value of $h = 2$. Some later developments brought more light to the problem of how single-strand lesions are deployed between strands of DNA molecules and their influence on DSBs formation [2] as well as the difference in the enzyme and particle action on DNA molecule. Moreover, it was found that the conformation of the plasmid molecule has a great impact on the yields of damage obtained from irradiation [3].

The earliest studies employed sucrose gradient centrifugation for breaks analysis and focused only on transitions from relaxed, R, to linear, L, DNA form. When a new experimental method, agarose gel electrophoresis, was brought in for analysis of DNA damage induced by radiation [4], the possibility of investigating the decrease in supercoiled, S, DNA levels arose. The relations between various topological forms of DNA as well as levels of damage that could be detected with this new method were derived. Further fragmentation of linear molecules was also investigated and the loss of linear molecules due to multiple DSBs (MDSBs) formation was described [5]. This fragmentation was also considered with respect to the DSB in DNA being a result of a multiple SSBs accumulation or by simultaneous cleavage of both strands by a damaging agent.

In all cases the experimental results obtained for various types of damaging agents showed some discrepancies in the determination of the h value. In addition, with development of the theory, more accurate modeling of experimental data was possible, leading to a correct description of the damage formation mechanism.

Nowadays more sophisticated computational models are in use. Due to their complexity it is now possible to deal not only with simple break formation, but also to account for the influence of irradiation of the DNA environment, i.e. living cell, and thus secondary species attack [6–9]. Moreover, an accurate examination of the mode of action of various incident particles can be performed at the same time [10, 11].

The very first attempts to describe transformations in DNA conformations upon damage turned out to give a solid base for SSBs and DSBs determinations and are continuously used for simple modeling of damage in experimental works dealing with DNA and protein damage induced by chemical agents [12], ions [13], OH radicals [14, 15], photons [16] or neutrons [17]. In this paper the most important early developments in DNA damage assessment theory will be presented and summarized.

2. Modeling of DNA damage

Very early studies [1] introduced a simple model for the assessment of the h parameter from the molecular weight decrease of enzymatically treated DNA molecules. The main disadvantage of this approach was that it takes an average number of DSBs per DNA molecule only. Also, an assumption was made, stating that all phosphodiester bonds in a DNA molecule are equally breakable. Such simplifications could be valid when breaks are created by enzymes, such as DNA-ase, but they are not valid when particles are the damaging agent. Therefore, in order to satisfy more general experimental conditions, more detailed modeling was required.

2.1. The Freifelder and Trumbo model [2]

In the model developed by Freifelder and Trumbo [2] the authors analyzed the transitions between R and L DNA forms, initiated by X-ray radiation. Both the average numbers of DSBs and SSBs were taken into account in order to determine the h value. The reasoning for this work was a mismatch between new experimental data and earlier theory. The discrepancy was attributed to the effects caused by obtaining lesions in DNA molecules with irradiation by high energy particles rather than enzymes. By analyzing the probabilities of DSB formation depending on the position of the first and all the following SSBs in both strands, a general formula for the probability that no DSBs will occur in the molecule, $\mathscr{F}(N)$ was derived. By solving this equation, the authors showed that a fraction of molecules, which does not suffer a DSB is

$$\mathscr{F} = \left[\frac{\mathscr{L}-\mathscr{R}}{\mathscr{L}}\right]^b = \frac{\mathscr{L}-b(2h+1)^b}{\mathscr{L}}, \tag{1}$$

where $b{=}\frac{1}{2}p_s$ is the average number of SSBs per strand, p_s is the average number of SSBs per double-stranded molecule, $\mathscr{L}$ is the number of phosphodiester bonds per single strand and $\mathscr{R}$ is the number of forbidden positions for next SSB formation.

Using the Poisson distribution of SSBs in the molecule to evaluate their number N and thus accounting for varying numbers of such lesions in molecules that are present in the sample, a more precise equation describing $\mathscr{F}$ was obtained

$$\mathscr{F} = \sum_{N=0}^{\infty} [\mathscr{F}(N)\mathrm{e}^{-p_s}(p_s^N/N!)], \tag{2}$$

where $\mathscr{F}(N)$ is a probability that N SSBs in a molecule are arranged so that no DSBs can be formed. The authors did not find this equation to be more precise when fitting their experimental data, in addition, the solution in computationally more difficult to find. Thus, the equation (1) was rearranged in order to obtain the value of h

$$h = \frac{\mathscr{L}}{2b}(1-\mathscr{F}^*) - \frac{1}{2}, \tag{3}$$

where $\mathscr{F}^*$ is the b-th root of $\mathscr{F}$. If h needs to be expressed in terms of p_d, the average number of DSBs per molecule, the calculations lead to

$$h = \frac{p_d\mathscr{L}}{2b^2} - \frac{1}{2} \qquad \text{or} \qquad p_d = \frac{b^2}{\mathscr{L}}(2h+1). \tag{4}$$

Equation (4) is equivalent to the formula derived in [1], given for a polydisperse system.

To account for different probabilities of damaging phosphodiester bonds by irradiation the authors discussed the likelihood of the SSB occurrence next to a specific DNA base. Assuming that the DNA sample used in the experiment contained molecules large enough to be considered possessing a random sequence of nucleotides in both strands and that the breaks can only occur adjacent to a purine, a quarter of the phosphodiester bonds could not be broken. Therefore, the probability that a phosphodiester bond will be broken, $b/\mathscr{L}$, should be taken as $b/(3/4\mathscr{L})$ instead. As the same reasoning would apply to the number of forbidden regions in the molecule, $\mathscr{R}$, thus their ratio in equation (1) remains unchanged.

2.2. The Blok and Loman model [18]

In some later work the damage yield was analyzed in terms of the action of secondary particles, like electrons or radicals that are formed in living cells upon irradiation. First, a formula for D_{37}, a 37 % survival dose where, on average, there is one lethal lesion present per one DNA molecule, was derived to yield

$$D_{37} = \frac{100}{rQ}(N_0 + C), \tag{5}$$

where N_0 is the DNA concentration (number of molecules per ml), r is the number of eV per gram per rad. Q and C are given by $Q = \sum_i \varepsilon_i G_i$ and $C = \frac{S}{Q}\sum_i \alpha_i \varepsilon_i G_i$, where G_i is the radical yield ($\mathrm{OH}^\bullet$, $\mathrm{H}^\bullet$ or e^-_{aq}, respectively), and ε_i is the efficiency of inactivation of a reaction between the i-th radical and the DNA molecule. Because the solution always contains impurity molecules that remove part of the primary radicals, the α_i parameter, which describes a ratio of rate constants of the i-th radical for reaction with impurity and DNA molecules, respectively, was introduced. Nonetheless, no allowance for impurities present in the solution that originate from DNA preparation was made. Furthermore, the authors assumed that these impurities can only act as radical scavengers. The authors did not observe a linear relationship between D_{37} and DNA concentration in their experimental results. The discrepancies between the derived equation (5) and experimental data were explained to be due to a secondary radical formation in the DNA molecule upon attack of primary radicals. The molecule itself would not suffer damage but would interact further with other, undamaged molecules in the solution.

The average number of single strand breaks present in the double stranded molecule, p_s, being a sum of such lesions present before, p_s^0, and after irradiation, p, in a molecule containing $\mathscr{L}$ base pairs was shown to be

$$p_s = p + p_s^0 = 2\mathscr{L}n, \tag{6}$$

where n is the probability that a break has occurred between two arbitrarily chosen neighboring bases in a strand. The authors determined the average number of DSBs per molecule, p_d, with respect to the average number of SSBs that appear in the double stranded molecule

$$p_d = \frac{(p+p_s^0)^2}{4\mathscr{L}}(2h-1) = \frac{p^2+2p_s^0 p}{4\mathscr{L}}(2h-1) + const. \tag{7}$$

If no SSBs are present in the DNA sample before irradiation, the constant in equation (7) is small and can be neglected, thus the relationship is similar to (4) given in [2]. The obtained relationship contains both linear and quadratic terms for SSBs appearance in the sample upon irradiation. Also, in case of lack of initial breaks the formula simplifies to a quadratic-only relationship and h can be easily calculated from the slope of p_d versus p_s plot.

The value of h obtained by the authors was very close to the ones obtained previously, but a mismatch between calculated and measured values of p_s^0 gave a reasonable doubt on the accuracy and the rationale of theoretical analysis presented by the authors. Also, the experimental data were showing a linear increase in p_d with p_s at low doses of radiation, instead of a quadratic one. This was explained by introducing two mechanisms of DSBs formation. It was postulated that at low doses DSBs are formed by single-hit events involving radical attack and, thus, the initial presence of SSBs is not required, whereas at higher doses the accumulation of SSBs will cause an increase in the number of DSBs as a primary mechanism.

This formula was later used by van der Schans [3] giving four times larger values of h when applied to his data for γ-irradiated DNA samples. A temporal local denaturation following SSBs appearance was given as a possible explanation.

2.3. The van Touw model [4]

The model presented by van Touw and coworkers [4] was developed to handle the data authors obtained from a newly developed method for DNA breaks analysis - agarose gel electrophoresis. In their experiments they could analyze not only the transitions between R and L forms of DNA, but also the loss of the initially supercoiled DNA. Contrary to gradient centrifugation techniques [3, 19], for the first time it was possible to follow dose-dependent quantitative changes in all three DNA forms since they got separated completely on the gel. All the previously derived models either did not account for the contribution of various mechanisms to DSBs formation or treated the formation of SSBs by simply averaging their amount between both strands in the double stranded molecule. According to the authors, such an assumption was not correct because it does not allow for overlapping of SSBs in both strands within the h distance. The formulas derived here took into account a binomial distribution of the SSBs between two DNA strands and the probability that regions with SSBs in opposite strands within h can overlap each other.

The authors analyzed the probabilities with which the molecules in an irradiated sample are affected by SSBs and DSBs formation. Also, a multiple hit DSB originating from two independently formed SSBs was distinguished. Moreover, the model allows predicting levels of potential SSBs that are not visible on the gel, but still present in DNA molecules. For all these cases a binomial distribution of probability of a hit was used and a Poisson probability of lesions distribution was applied. The analysis led to the conclusion that a probability, w, that a SSB in one strand will find a SSB in the other strand within a distance h is $2a/\mathscr{L}$. Thus, a fraction of molecules, f, of contour length $\mathscr{L}$, that has, by single hits, n_d DSBs and n_s SSBs and additional n_{ds} DSBs as a result of n_s SSBs was derived to be

$$f(p_d, p_s, 2h/\mathscr{L}; n_d, n_s, n_{ds}) = P(p_d; n_d) \cdot \left\{ P(p_s; n_s) \cdot \sum_{k_s=0}^{n_s} [B(n_s, 1/2; k_s) \cdot B(m, w; n_{ds})] \right\}. \tag{8}$$

Noticing that f given by (8) is a multivariate distribution function, a relationship between p_{ds} and p_s (in

agreement with [18]) was also obtained

$$p_{ds} = \left(\frac{p_s}{2}\right)^2 \cdot \frac{2h}{\mathscr{L}}. \tag{9}$$

The p_s and p_d parameters, representing the average numbers of SSBs and DSBs that originated from a single-hit event per molecule, can be related to the radiation dose, D, in the following way

$$p_s = p_s^0 + D/D_{37s}, \tag{10}$$

$$p_d = p_d^0 + D/D_{37d} = p_d^0 + \varepsilon p_s, \tag{11}$$

where p_d^0 and p_s^0 represent numbers of SSBs and DSBs in molecules at zero dose, respectively, and D_{37s} and D_{37d} are doses, at which there is, on average, one single-hit SSB and one single-hit DSB per DNA molecule, respectively. The ε parameter expresses the efficiency with which a single-hit DSB is created over a SSB.

From (8) the authors derived a set of formulas allowing to follow the changes in S, R and L forms of DNA. For fraction S that has no SSBs ($n_s = 0$) and DSBs ($n_d = 0$) and thus no multiple-hit DSBs ($n_{ds} = 0$)

$$S(n_d, n_s, n_{ds} = 0) = P(p_d; 0) \cdot P(p_s; 0). \tag{12}$$

In case of fraction R that has at least one SSB ($n_s \geq 1$), no DSBs ($n_d = 0$) and thus no multiple-hit DSBs ($n_{ds} = 0$), a following relation was derived

$$R(n_d, n_{ds} = 0, n_s \geq 1) = P(p_d; 0) \cdot \left\{ \sum_{n_s=0}^{\infty} \left[P(p_s; n_s) \cdot \sum_{k_s=0}^{n_s} \left(B(n_s, 1/2; k_s) \cdot B(m, w; 0) \right) \right] - P(p_s; 0) \right\}. \tag{13}$$

The last fraction, L, is the fraction, which has only one DSB created either by single- or double-hit ($n_d + n_{ds} = 1$)

$$\begin{aligned} L(n_d + n_{ds} = 1) = P(p_d; 1) \cdot \sum_{n_s=0}^{\infty} \left\{ P(p_s; n_s) \cdot \sum_{k_s=0}^{n_s} \left[B(n_s, 1/2; k_s) \cdot B(m, w; 0) \right] \right\} \\ + P(p_d; 0) \cdot \sum_{n_s=0}^{\infty} \left\{ P(p_s; n_s) \cdot \sum_{k_s=0}^{n_s} \left[B(n_s, 1/2; k_s) \cdot B(m, w; 1) \right] \right\}. \end{aligned} \tag{14}$$

The authors derived also a simplified set of equations describing quantitative changes in all three DNA fractions upon irradiation and lesions formation

$$S = \mathrm{e}^{-p_d} \cdot \mathrm{e}^{-p_s}, \tag{15}$$

$$R \simeq \mathrm{e}^{-p_d} \cdot \left[\mathrm{e}^{-p_{ds}} - \mathrm{e}^{-p_s} \right], \tag{16}$$

$$L \simeq (p_d + p_{ds}) \cdot \mathrm{e}^{-p_d} \cdot \mathrm{e}^{-p_{ds}}. \tag{17}$$

These equations are valid only when numbers of p_s and $2h/\mathscr{L}$ are low and p_d/p_s is high. Otherwise some inconsistency at higher doses, i.e. higher p_s numbers, can be seen between equations (12)–(14) and (15)–(17).

The approach taken by the authors allowed for the first time to determine numbers of SSBs, DSBs, loss of initial S form and the distance h, required to create a DSB from two independent SSBs in irradiated molecules. The distance h, calculated using this model, was shown to be approximately 30 bp. Such a large discrepancy with values found in the literature (see section 2.2) was ascribed to a difference in the DNA preparation method rather than a result of more accurate modeling. Although the authors provided a

very detailed set of equations describing changes in topoisomeric forms of DNA (12)–(14), unfortunately the formulas did not include the evolution of the fragmented form, F, which certainly appears at higher doses of radiation.

Nonetheless, it was also possible to follow the time evolution of three main DNA topoisomeric forms. The simplicity of the new experimental method – agarose gel electrophoresis – made it being widely used by scientists in radiation studies.

This model was later modified by Hempel and Mildenberger [20], who noticed that the number of DSBs, p_d given by (11) should include a factor for p_{ds} as both types of damage will create an L form of DNA

$$\widetilde{p}_d = p_d + p_{ds}. \tag{18}$$

The h value obtained from fitting to the experimental data was much higher than reported by others, reaching 60 bp. It was doubted that such long distance between SSBs would cause enough instability in the molecule in order to create a DSB. The authors had also noticed that from the simplified set of equations (15)–(17) one can derive another formula describing changes in the levels of DSBs, namely

$$\widetilde{p}_d = \frac{L}{S+R}. \tag{19}$$

The advantage of this modification was that only the relative fluorescence measurements of the gel had to be performed in order to assess levels of DNA damage.

2.4. The Cowan model [5]

Simultaneously, another model, based on earlier work [2, 21], was derived. The damage to the DNA sample was introduced by nicking and cutting enzymes that can mimic breaks appearance in DNA upon irradiation, allowing to fully control damage induction. Apart from managing the transitions between S, R and L DNA forms, the authors also considered various cases, including further fragmentation of linear DNA due to MDSBs formation and thus appearance of the F form. In addition, a situation, where not 100 % of the starting material is in a supercoiled form was discussed.

First, the case with only the single stranded nicking being the damaging agent was considered. Changes in levels of the supercoiled DNA form were assumed to follow an exponential loss of the starting material with time, t

$$S(\mu) = \mathrm{e}^{-\mu}. \tag{20}$$

with $\mu = \lambda \cdot t$, where λ is a proportionality constant. The μ parameter is the number of nicks created in a molecule up to time t and can be considered as a 'nicking dose'. Derivation of other formulas, describing changes in levels of R and L forms, required more advanced mathematics and led the authors to the following relations

$$R(\mu) = 2\mathrm{e}^{-\mu/2} - 2\mathrm{e}^{-\mu} + \mu X, \tag{21}$$

$$L(\mu) > q^{-1}\left(\mathrm{e}^{\mu q/2} - 1\right)\left(\mu X - Y + \mathrm{e}^{-\mu/2} - \mathrm{e}^{-\mu}\right) \qquad \text{and} \tag{22}$$

$$L(\mu) < \mu\left(2 - q\mu\right)^{-1}\left(\mu X - Y + \mathrm{e}^{-\mu/2} - \mathrm{e}^{-\mu}\right), \tag{23}$$

where

$$X = \sum_{k=1}^{\infty} \mathrm{e}^{-\mu(1+kq)/2}[\mu(1-kq)_+/2]^{2k-1}/(2k!) \qquad \text{and} \tag{24}$$

$$Y = \sum_{k=1}^{\infty} \mathrm{e}^{-\mu(1+kq)/2}[\mu(1-kq)_+/2]^{2k-1}[2k + \mu(1-kq)/2]/(2k!). \tag{25}$$

The q parameter denotes the size of the taboo zone – the area, where no further breaks can form. The MDSBs were simply assessed to be the rest of the material missing from the quantification of the three main forms with respect to the initial conditions

$$F(\mu) = 1 - S(\mu) - R(\mu) - L(\mu). \tag{26}$$

When an additional factor, causing a DSB formation simultaneously to SSBs appearance with efficiency ϕ (cutting dose), proportional to t via constant ρ, was introduced, the equations (20)–(23) took the following form

$$S(\mu,\phi) = \mathrm{e}^{-\phi} S(\mu), \tag{27}$$

$$R(\mu,\phi) = \mathrm{e}^{-\phi} R(\mu), \tag{28}$$

$$L(\mu,\phi) = \mathrm{e}^{-\phi}[L(\mu) + \phi\{S(\mu) + R(\mu)\}]. \tag{29}$$

In the situation, where the cleaving mechanism dominates over the single strand cut, it was possible to simplify the theory to

$$S(\mu,\phi) = \mathrm{e}^{-(\mu+\phi)} \tag{30}$$

$$R(\mu,\phi) = \left(1 - \mathrm{e}^{-\mu}\right)\mathrm{e}^{-\phi} \tag{31}$$

$$L(\mu,\phi) = \phi \mathrm{e}^{-\phi} \tag{32}$$

$$F(\mu,\phi) = 1 - \mathrm{e}^{-\phi}(1+\phi). \tag{33}$$

By dividing equations (30)–(32) by $1 - F(\mu/\phi)$, the authors obtained a set of formulas that correspond to the bands on gel and thus values for S, R and L can be obtained directly. In such case also μ and ϕ can be determined as

$$\phi = L'/(1 - L'), \tag{34}$$

$$\mu = -\ln[S'(1+\phi)], \tag{35}$$

where S' and L' are the relative proportions of S and L forms. Accounting for the fact that not 100 % of the starting material being in supercoiled form increased to the values of μ and ϕ by starting nicking and cutting doses μ_0 and ϕ_0

$$\mu = \mu_0 + \lambda t, \tag{36}$$

$$\phi = \phi_0 + \rho t. \tag{37}$$

The last situation that was analyzed was the case when both mechanisms were taking place together with topoisomerase I action that unwinds supercoiled molecules leaving them in an R form with no breaks. Therefore, it was necessary to introduce another parameter, θ, describing the rate of topoisomerase I interaction with DNA. This parameter is analogous to μ and ϕ, except that the interaction has an effect only if it is the primary one of all three. Equations (26) and (29) remain unchanged, whereas (27) and (28) take the following form

$$S(\mu,\phi,\theta) = \mathrm{e}^{-\theta} S(\mu,\phi), \tag{38}$$

$$R(\mu,\phi,\theta) = R(\mu,\phi) + \left(1 - \mathrm{e}^{-\theta}\right) S(\mu,\phi). \tag{39}$$

As previously, a simplified theory for the case of DNA cleavage being the predominant mechanism was derived. The obtained equations were the same, except for μ being replaced by $\mu + \theta$.

From plots of molecular proportions of different DNA forms, obtained for varied values of all three parameters, the authors concluded that if only the nicking mechanism of DNA damage is involved, the DSBs start appearing when almost all of the S form of DNA is lost. When, in addition, a cleaving mechanism is involved, even a small increase in the cleaving rate ϕ would cause much faster degradation of the starting material; the simplified theory can only be used when DNA cleaving is truly the

predominant mechanism. Their final finding was that if in the initial sample there are some relaxed but not nicked molecules or the molecules, initially supercoiled, undergo unwinding before nicking and cleaving, the loss of the supercoiled material is much faster, yet the topo-enzyme plays no role once all supercoiled molecules are lost. In comparison to the earlier model [2], the authors used their exact formula (21) and concluded that the oversimplified approach used previously underestimates the proportion of relaxed molecules being lost due to DSBs formation from multiple SSBs.

3. Summary

In this paper a few initial statistical models aiming at describing the distribution of lesions in DNA molecules, caused by damaging agents like enzymes, ionizing radiation or secondary species attack, are presented. With the development of experimental techniques, allowing for the separation of main topological forms of DNA, there was an urge to improve models in order to account for new phenomena that could then be observed. The earliest attempts were developed further to account for more topological DNA forms as well as for various conditions under which damage can be induced. The value of h parameter varies between models and experiments, still raising the issue of the length of the h that is necessary to create a DSB from two SSBs.

The distinction between the mode of action of an enzyme and radiation on a DNA molecule permitted to develop independent methods for description of topological changes in DNA. The accurate description of dynamical changes in DNA conformations upon the action of the damaging agent allows an assessment of the efficiency of the agent in damaging the DNA molecule that is an important issue in cancer therapy. The most advanced models of those presented here have laid the foundation for more advanced modeling developed recently. Nonetheless, they are still in use, aiding the analysis of experimental data and dealing with outcomes of the radiation-induced damage by electrons, photons or ions.

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